TRANSACTIONS OF THE WAGNER FREE INSTITUTE OF SCIENCE

OF

PHILADELPHIA

VOL. IX—PART I

MAY, 1919

WAGNER FREE INSTITUTE OF SCIENCE
MONTGOMERY AVE. AND SEVENTEENTH ST.
PHILADELPHIA

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THE VEGETATION OF THE HACKENSACK MARSH: A TYPICAL AMERICAN FEN

By JOHN W. HARSHBERGER and VINCENT G. BURNS*

INTRODUCTION (V. G. B.)

THE Hackensack River, which may be regarded as a branch of the Passaic, rises in the northeastern corner of the State of New Jersey, and drains a region of considerable extent lying along the west slopes of the Palisade Range. From the town of Hackensack south to the outlet in Newark Bay the river occupies a valley approximately four miles in width and eight miles in length, which is largely filled by a brackish tide-water marsh. On the west the marsh is separated from the valley of the Passaic by a long, low ridge of reddish-brown sandstone (Newark System of Triassic), and on the east a parallel ridge of igneous rock (Palisade Diabase of Triassic) separates it from the Hudson River. Outcroppings of the latter—igneous intrusion—are found in the center of the valley in the form of two large rock masses, known as Snake Hill and Little Snake Hill (Fig. 1). Owing to the scarcity of connected outcrops over the entire area of the Hackensack Valley, no definite stratigraphic succession of the rocks underlying the marsh has been determined up to the present time. However, it is stated in the Geologic Atlas of New Jersey, published in 1908, that the greater part of the Hackensack marsh is known to lie "in a deep depression excavated mainly in shales which have been reached by some of the wells." Numerous borings about Newark, Hackensack, and neighboring points have given information concerning the layers which occur above the underlying rock. Data obtained from records of these borings, in the Geologic Atlas of New Jersey (1908) and from Mr. Hewitt Crosby, Reclamation Engineer of New York City, make possible the following interpretations of the foundation of the marshes.

It is definitely known that the marsh is underlaid by a layer of Triassic red shale, because this rock has always been reached wherever deep borings have been made. That this shale constituted the main part of the exposed rock in the region of the marsh just prior to the Pleistocene Glaciation seems

* The separate parts of this contribution to botanic science are indicated by the initials of author contributing that part, viz., J. W. H. and V. G. B.

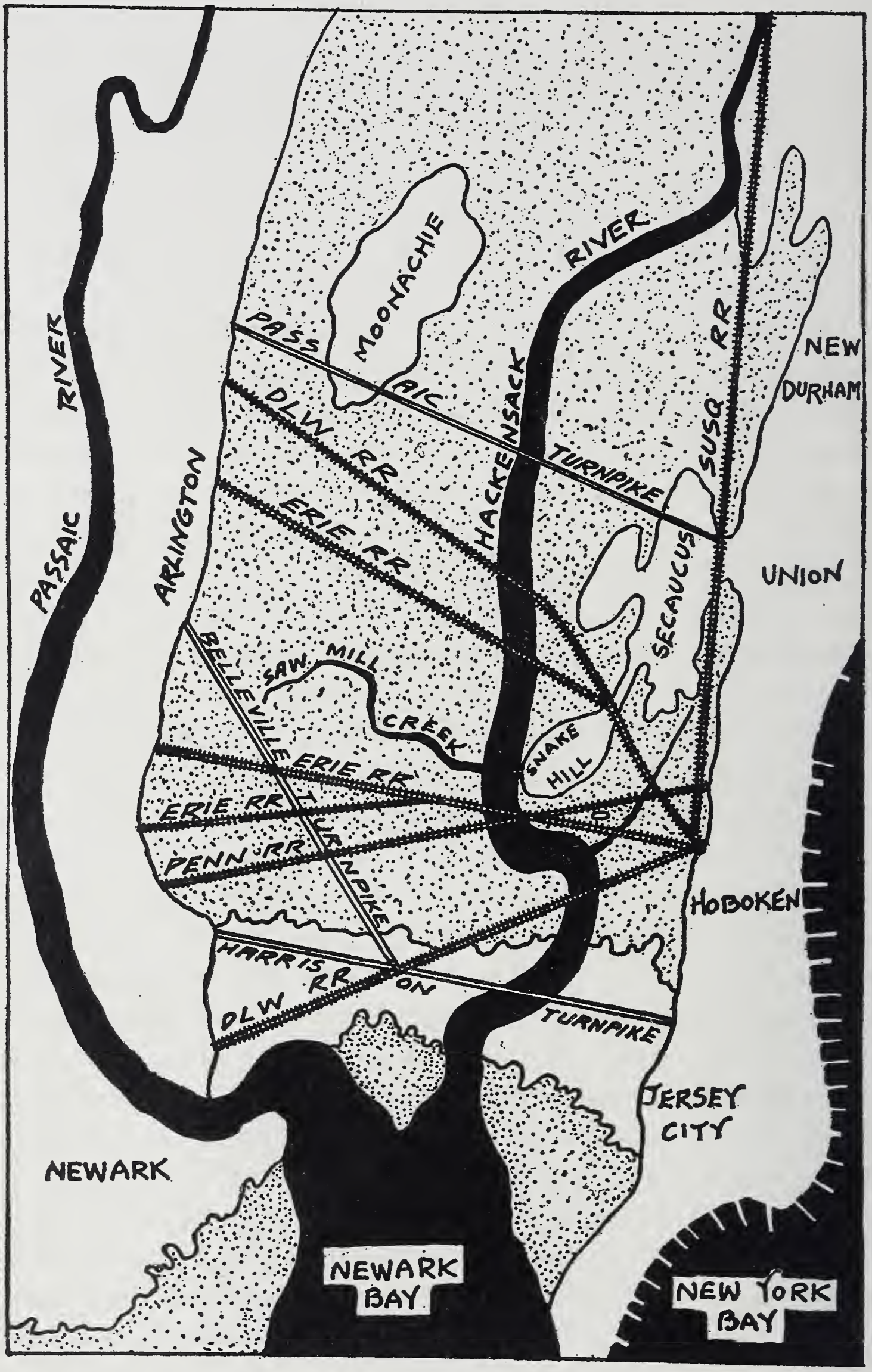

FIG. 1.
Sketch map of the Hackensack Marsh, New Jersey. Vincent G. Burns.

evident from the fact that at the present time the glacial drift lies just above it. The drift is mixed with and covered by a deep stratum of laminated clay which contains gravel, boulders, and calcareous nodules in abundance. The composition of the clay and its position just above the glacial drift lead one to believe that it was deposited in standing water left by the retreating Pleistocene ice-sheet. The thickness of this clay stratum varies from 85 to 250 feet, the greater thicknesses being due to the presence of large quantities of drift.

Just above the laminated clay occurs a thin layer of alternating sand and yellow loam, averaging 2 to 3 feet in thickness. The loam corresponds exactly to that found exposed at many places adjoining the Hackensack marsh, and it is quite probable that the loam, now covered, was at one time continuous with that exposed in neighboring regions. The presence of the sand at different places is puzzling. It may be the remains of the old sandy sea-beach upon which the present marsh was built by the action of the tide, but this is not at all certain (Fig. 2).

Above the shallow layer of sand and loam there is a much deeper stratum of black, soggy muck which ranges from a depth of only 1 foot to 70 feet in some places. This is chiefly a mixture of dark silt, organic matter, water, and gases (CH_4, CO_2, H_2S, etc.), and probably owes its origin to long action of the tidal water in depositing silt and also to the accumulation of plant remains.

Perhaps, also, after the final withdrawal of the great ice-sheet from the glaciated regions of America, a shallow inland bay or arm of the sea was left where the Hackensack marsh now is. The flat margins were, no doubt, wet enough to support marsh vegetation, and as the time went on this vegetation came to fill the bay, leaving open the tortuous tidal channels, or creeks, which intersect the surface of the present marsh. The fact that stumps with roots of the white cedar, *Chamæcyparis thyoides,* are found imbedded in the muck indicates (Fig. 3) that in recent times the marsh surface in places was above tide-water and covered with groves of trees,* and for the reason that this tree has been reported near Newark by W. M. Wolfe. The obliteration of most of this acid swamp was brought about probably either by a resinking of the shore

* Cf. somewhat similar conditions of the encroachment of salt marshes on Chamæcyparis trees in Massachusetts, viz., *Bartlett, Harley H.:* The Submarine Chamæcyparis Bog at Woods Hole, Massachusetts, Rhodora 11: 221–235, December, 1909.

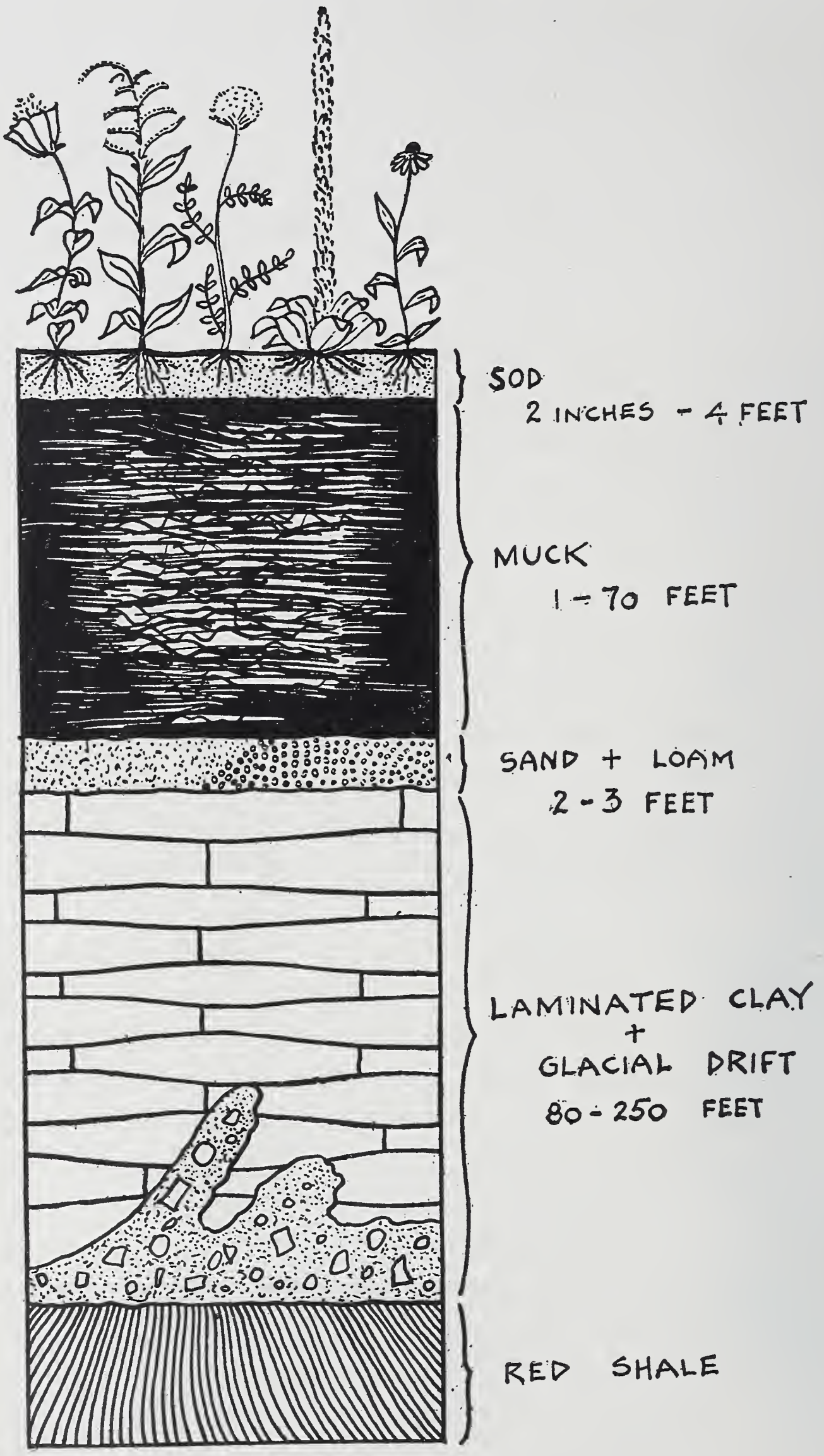

FIG. 2.

Cross-section of the soil layers of the Hackensack Marsh. Vincent G. Burns.

line or by a change in the tidal levels, which introduced brackish or saline conditions again.

Overlying the muck and covering the marsh surface is a dense mat of roots, plant remains, and decaying organic material, which forms a sod ranging from 2 inches to 4 feet in thickness (Fig. 2). This sod, when dry, is extremely hard and compact, and for this reason is used advantageously in dike-building.

COMPARATIVE STUDY OF FEN VEGETATION (J. W. H.)

Ecologic research has reached such a stage in America that it is profitable to compare the phytogeographic formations of North America with similar ones of other lands. This comparative study is necessary in order to give us clearer views of the causes underlying the distribution of plants with respect to their habitat conditions. The author has recently paid considerable attention to this phase of ecologic research, and has printed one paper dealing with "American Heaths and Pine Heaths." He has had accepted for publication another comparative study of "Alpine Fell-fields in Eastern North America," and he has the photographs and material assembled for another contribution on "The Influence of Slope Exposure in Plant Distribution." The present account of the ecology of the Hackensack Marsh, written in conjunction with the floristic studies of Vincent G. Burns, is a fourth contribution to such comparative ecology.

The vegetation of the Hackensack Marsh constitutes a number of plant formations, two of the most important being the salt marsh formation and the fresh-water marsh formation. In England the type of fresh-water marsh represented in America by the part of the Hackensack Meadow controlled by fresh water is called a fen. It is important, as ecologists, for us to have clear concepts of the character of the units of vegetation in this and other countries before we attempt to describe them. This desideratum is reached in part by comparing the words used in different places for the same phytogeographic formation. Sometimes the popular usage runs parallel with the exact scientific application of the name. At other times it does not become applicable. The terms "marsh" and "swamp," in common language, are frequently used interchangeably. But phytogeographically speaking, marshes and swamps are quite different, physiognomically and floristically. The two terms "moor" and "fen" are often confused in England. A distinction must be made

between them. There are great tracts of peat laid down in the upper part of old estuaries, and around fresh-water lakes, which are called fens. Such terms as the French "Le Marais immerge," "Le Marais lacustre," the Bohemian "Slating," are akin to that of fen. The soil of a fen is a muck, rather than a peat, fed by telluric water with an alkaline reaction and relatively rich in mineral salts. This character of the soil has a profound effect on the vegetation.

ENGLISH FENS

Considerable attention has been given of late years, since the ecologic awakenment of English botanists, to the vegetation of fens or reed swamps (reed marshes). One of the earliest accounts is by Yapp, on Wicken Fen, not far from Cambridge, in the region of the Wash.* The dominant herbaceous plants of this fenland are tall, reedy grasses, sedges, and rushes growing out of the wet muck. The raised parts of the fen can be distinguished at a distance by the plants growing on them. Thus, toward the end of July many of these banks are easily recognized by the masses of flowering *Spiræa Ulmaria* and associated species. Most of the plants that grow in the wet have creeping stems and stiff pointed shoots that can easily force their way up through the overlying muck. The roots of fen plants are placed more or less horizontally. The stratification in the vegetation of this marsh has been described in a later paper by Yapp, where emphasis is placed upon the fairly uniform facies of the vegetation and upon the different types of plants which enter into competition in the marshland.

Marietta Pallis furnishes a chapter in the "Types of British Vegetation" (1911: 214–234) on the fenlands of the Broads in the inner valleys of East Norfolk and East Suffolk. The Bure valley fen shows in summer a dense growth of grasses and sedges, such as *Phragmites communis*, *Molinia cærulea*, *Cladium Mariscus*, while the Yare valley fen at the same season of the year is a wild flower garden forming a different type of marshland. The Bure fen has only one species dominant, viz., *Phragmites communis*. Some of the fenland is characterized by the presence of trees and shrubs, which form the fen thickets, or carr.

Moss† emphasizes the chemical character of the soil as of importance for

* *Yapp, R. H.:* Sketches of Vegetation at Home and Abroad: IV: Wicken Fen. The New Phytologist, VII: 61–81, Feb. and March, 1908; Annals of Botany, XXIII: 275–319, Apr., 1909.

† *Moss, C. E.:* Vegetation of the Peak District, 1913: 168–171; Jour. of Ecol., VI: 53–74.

the peaty tracts of eastern England, which are characterized by alkaline waters, are spoken of usually by the local inhabitants as "black fens," or simply fens characterized by alkaline waters and by a high, soluble mineral content—especially by a high lime content. Accordingly, the vegetation of acidic peat may be said to belong to the moor formation, and that of the alkaline peat (muck) to the fen formation. The lists of plants for the two formations bring out in a marked degree these fundamental differences.

The aquatic and marsh vegetation of Esthwaite Water has been investigated recently by W. H. Pearsall,* who shows that the conditions of sedimentation influence the character of the associations. The reed swamps of the area of rapid sedimentation are peculiar in the absence of *Scirpus lacustris*, the presence of *Typha latifolia*, and the abundance of herbaceous species. He describes the mixed fen associes, which succeed reed swamp, as also the vegetation of the areas of moderate and slow sedimentation.

FENS OF SWITZERLAND

For Switzerland, Früh and Schröter, in a monumental contribution, describe the flachmoor (reed marshes) of that country. They recognize a number of different types which owe their character to the plants which are associated. The roseau (Fr.), cannetta (Ital.), reed (Engl.), is the tallest and most abundant constituent of the fens of Switzerland. Its rhizomes spread horizontally considerable distances, forming a mat which binds the mud and slimy ooze into a rather firm mass. It contributes largely to the formation of the muck. The nodes of the creeping stem give rise to upright halms and roots. In such Swiss lakes as Lake Zug, there are scattered areas of *Phragmites communis* along the lake shores. Here it is associated with *Scirpus lacustris*, which is codominant with it. The width of the areas where these two plants are found depends on the depth of the water, which is shallow, especially along the west shores of the lake.

PAPYRUS MARSHES IN SICILY

Near Syracuse in Sicily are two small rivers, the Anapo and the Ciani, which are characterized by growths of the papyrus (*Cyperus Papyrus*), the physiognomy of which resembles that of the fenlands of England and elsewhere, and in the broad sense the papyrus marsh may be included in the fen-

* *Pearsall, W. H.:* Jour. of Ecol., VI: 75–83, March, 1908.

land formations. The halms of this classic plant of Egypt reach a height of over 3 meters. Associated with the papyrus is the Spanish reed (*Arundo Donax*), the largest grass of Europe.

REED MARSHES OF CENTRAL AND EASTERN EUROPE

Reed marshes are found in Saxony in several facies, one of which corresponds with the fen formation of England. The reed marshes of the Carpathian region of Europe occur in the Hungarian Plain, along the streams, and similar marshes exist in Illyria.

The Plav of Rumania, according to Miss Pallis,* is a floating fen formed almost entirely of living reed, *Phragmites communis β flavescens*. There are three more or less well-marked growth stages of the reed grass, namely: the open, the closed reed, and, in deep waters, Plav. The open reed marsh is the stage at which the growth of the reed shoots is as yet sparse along the edge of the lake or a stream, and the plants are fixed. Closed reed develops from open reed marsh automatically, and in time becomes Plav. Much soil is held by individual tussocks and by closely growing assemblages of tussocks. Plav, when newly detached, does not differ from closed reed marsh, except in that it floats.

One plant, *Typha angustata*, competes successfully with the reed in the delta of the Danube River, though it probably never supplants the reed absolutely, but merely inhibits its growth for a time. In the delta, *Phragmites* invades deeper water than *Typha*, hence it is only in shallow water that they enter into competition. *Cladium Mariscus* also is found in the Danube fens.

ASIATIC FENLAND

In central Asia, *Phragmites communis* is found about nearly all of the rivers and lakes. The fens of eastern Turkestan, as at Kerija Darja, are the refuge places for the wild birds and wild pigs of that country. About Lop Nor, a lake in Chinese Turkestan, which is undergoing desiccation and is only a few feet deep, there are unprecedented growths of this reed grass, which, arching over the open channels of water, form tunnel-like passages for the movement of the native boats. Reed marshes are found not uncommonly in China (west of Shanghai), in Japan, about lakes Biwa and Chuzen.

* *Pallis, Marietta:* Structure and History of Plav: The Floating Fen of the Delta of the Danube. Journ. Linn. Soc., Botany, XLIII: 233–290, with plates and map.

FENS OF AFRICA

Typical fen vegetation, with *Phragmites communis* prominent, is found in Africa about Lake N'gami, at least 6 to 7 kilometers broad, and in Togoland, also about Lake Victoria Nyanza. The vleys of South Africa are characterized by broad bands of *Juncus maritimus*, associated with the two South African cattails, *Typha australis* and *T. capensis*, while *Phragmites communis*, which plays a prominent rôle in other parts of Africa, is sparingly found, or entirely replaced by *Cladium Mariscus*, associated with the calla-lily, *Zantedeschia æthiopica*, which, with its white spathes, enlivens the South African fenland in winter, while in summer water-lilies in flower are conspicuous in the open places of the reed marshes. Vleys (Vleis) are found in South Africa, according to Bews,* wherever a depression in the ground checks drainage, such areas being known as "flushes." Few cover more than an area or two of ground. The vegetation varies—(1) according to the amount of water present, and (2) according to the degree of stagnation of the water.

SUDD VEGETATION

Much has been written about the vegetation of the marshes of the Upper White Nile,† where vast masses of floating plants are moved hither and thither and block the waterways by forming dams (Arab, "sudd") across them. The mouth of the Bahr el Jebel, near Lake No and 627 miles above Khartoum, may be taken as the northern gate of the Sudd region—a vast country of marshes. The chief sudd-formers are *Cyperus Papyrus*, *Panicum pyramidale*, *Phragmites communis*, *Typha australis;* between them, as floating plants, occur the water-soldier, *Pistia Stratiotes*, *Azolla nilotica*, while the mass of plants forming the Papyrus fringe are bound together by numerous twiners and climbers. Toward Hillet en Nuer the banks are better defined and the papyrus is replaced by *Phragmites communis*, while *Panicum pyramidale*, called "Om-Suf," or "mother-of-wool," by the Arabs, on account of the irritant hairs at the base of the leaves, has the faculty of growing both in shallower and deeper water than the Papyrus, and not only occupies the land which is uncovered at low Nile, but forms a fringe in front of the Papyrus in the bed of the channel.

* *Bews, J. W.:* The Vegetation of Natal. Annals of the Natal Museum, II, Pt. 3: 320, May, 1912; Types of Vegetation in South Africa. Journ. of Ecol., IV: 147, Dec., 1916.

† *Brown, A. F.:* Some notes on the "Sudd" Formation of the Upper Nile. Journ. Linn. Soc., Botany, XXXVII: 51, 1904–1906.

NORTH AMERICAN FENS

The American counterparts of the fen formation of England and other parts of the world have been studied in a number of localities, but we lack ecologic details for a large number of areas of marsh which belong to this category. About the head of Cayuga Lake, in Central New York State, is found Renwick Marsh, near Ithaca. The largest association of plants in this marsh consists of two predominating species of cattail, *Typha latifolia* and *T. angustifolia*, with the former more abundant. The two plants seldom mingle and are associated with few other plants. The species of *Typha* reach a height of 8 to 9 feet, and the growth in height is completed by the latter part of June or early July. This association furnishes the optimum conditions for certain birds. Such are the red-winged blackbirds,* coots, Florida gallinules, and the least bittern.

Skokie Marsh is associated with Skokie Stream along the west side of Lake Michigan in Illinois. The reed-marsh association is the principal one of the marsh. Within the associations of *Nymphæa* and *Castalia* almost pure growths of *Typha latifolia*, *Sparganium eurycarpum*, *Scirpus fluviatilis*, *S. validus* occur, associated with scattering plants of *Sagittaria latifolia*, *Sium cicutæfolium*, *Dulichium arundinaceum*, and *Decodon verticillatum*. In certain parts of the reed marsh, at stations slightly less hydrophytic, *Phragmites communis* grows. Elsewhere there are scattered patches of *Iris versicolor* and *Acorus calamus*.

Sherff† has studied in a detailed manner and has made careful drawings of the under surface stems and roots of the typical marsh plants which come into competition. He finds that two or more species may be associated harmoniously, because—(1) their subterranean stems may be at different depths; (2) their roots may thus be produced at different depths; (3) even where the roots are produced at the same depth, they may make unlike demands on the soil.

Wild rice, *Zizania aquatica*, is a tall-growing marsh grass found in especially pure growths in the ponds and lakes characteristic of Wisconsin and Minnesota, where the *falle avoine* has been from the earliest times gathered

* *Allen, Arthur:* The Red-winged Blackbird: A Study in the Ecology of a Cattail Marsh. Proc. Linn. Soc. of New York, 1911–13: 43–128.

† *Sherff, Earl E.:* The Vegetation of Skokie Marsh. Bot. Gaz., LIII: 415–435, May, 1912.

by the Indian for the nutritious grains.* The physiognomy of these marshes, when viewed from a distance, is that of the typical reed marsh, and frequently, as will be shown subsequently, *Zizania aquatica* is associated with the typical fen plants and can with propriety be included with them.

Typical fens are found in two places in California. Klamath Lake is situated in northeastern California, on the Oregon boundary line. Its shallow water permits a great growth of tule, *Scirpus lacustris* var. *occidentalis*, which has stout creeping root-stocks and triquetrous stems, 2 to 5 feet tall. Rushes grow with the tule, and these plants together fringe the lake shore, in places expanding to a width of several miles. They also form islands varying in size from a few square yards to many acres in extent.†

The most extensive tract of fens on the Pacific coast follows the course of the Sacramento River for a distance of 150 miles on either side. Here *Scirpus lacustris* var. *occidentalis* chokes the marshland, associated with *Scirpus tatora*. Islands occur, formed of muck, and they are separated from each other by tortuous channels. Annuals here are generally 4 to 6 feet in height, and plants 8 to 12 inches high in dry soil here double their size.

The Everglades of Florida is an immense grassy marsh, or fen, covered in the wet season—June to November—with water to an average depth of 26 inches, stretching on all sides to the horizon, and relieved in some places by clumps of bushes or low trees (carr), and characterized by lagoons, channels, or slues of open water, or filled with various aquatic plants. It extends south toward Cape Sable, from the southern end of Lake Okeechobee. The soil is a black muck (2.5 to 10 feet deep), overlying the limestone rocks which form the bottom and sides of the basin in which the fenland occurs. The whole area is covered with a rank growth of a coarse sedge, 8 to 10 feet tall, having leaves with a fine edge, like a saw, hence the common name.‡ The saw-grass, *Cladium effusum*, arises from a root-stock with matted roots. It forms exclusive growth of such density as to become impenetrable, but with open stretches of clear water, covered at places with water-lilies and pickerel weed. Islands of bushes and trees (English carr) are scattered over the sur-

* *Jenks, Albert E.:* The Wild-rice Gatherers of the Upper Lakes. Report Bureau of American Ethnology, 19, pt. 2: 1019–1133 (1897–98).

† *Chapman, Frank M.:* The Habitat Bird Group, Guide Leaflet No. 28, American Museum of Natural History, Feb., 1909, pages 38 and 39.

‡ *Harshberger, John W.:* The Vegetation of South Florida. Trans. Wagner Institute, VII, Pt. 3: 155–166, Oct., 1914.

face of the saw-grass marsh (saw-grass fen). The Everglades, covering an area of 4000 square miles, or 2,560,000 acres, is the largest fresh-water marsh (fen) in North America, lacking the typical fen plants of other parts of the world, but making up in physiognomic aspect for the absence of such plants as *Phragmites communis*, *Typha latifolia*, and other plants by the presence of other grasses and sedges of similar botanic aspect.

HACKENSACK MARSH

The traveler between Philadelphia and New York, and the New York commuter who lives in New Jersey, see a great stretch of green flatland, with here and there the top of a fisherman's cabin, or the black mast of a catboat above the cattails and reed grasses over seven feet tall. Great factories are ever reaching on these marshes from all sides, and railroads cut them up into smaller and smaller areas of undisturbed vegetation. From a distance there is no trace nor sign of the many little hammocks that are found in the marsh, nor of the tidal creeks and estuaries which run in many directions. All that is noticeable in this general view is the unbroken verdure of the tall, reed-like plants, continually billowed by the passing breeze (Fig. 5).

The vegetation of the Hackensack fen may be divided into three formations, viz., the salt-marsh formation, the fresh-water marsh (fen) formation (Figs. 4, 5), and the marsh thicket (carr) formation (Figs. 7, 13). A bog formation (not studied) probably exists in the northern part of the region. Its investigation would probably give interesting details as to the succession of vegetation in the marshes.

These associations are well characterized, and their geographic location depends on the location of the marsh with reference to salt water, the highland on three sides of the marshy region, and on the direction of the fresh-water streams which run across the lowlands.

Salt Marsh Formation.—The natural undisturbed surface of the salt marsh of the Hackensack Meadow is fairly uniform in character. It is found at the mouths of the creeks and rivers which intersect the region, and around the margins of the lagoons and estuaries, forming extensions landward of Newark Bay. The influence of salt water is felt some distance above Newark Bay, and the tidal channels permit the entrance of sea water, so that

FIG. 3.
Holes in muck of Hackensack Marsh from which stumps of white cedar in the background have been extracted, July 15, 1916. J. W. H.

FIG. 4.
Tall Cattails, *Typha latifolia*, with Mullein, *Verbascum Thapsus*, Hackensack Marsh, July 15, 1916. J. W. H.

daily the surface of the salt marsh is partly or wholly flooded with salt or brackish water.*

The outer margin of the salt marsh, where it touches the open lagoon, or the tidal thoroughfare, is fringed with a broader, or a narrower, strip of the tall salt grass, *Spartina glabra* var. *pilosa*. Back of this strip, whose width depends on the slope and the height to which the tide rises, we find the rush salt grass, *Spartina patens*, which grows at a slightly higher tidal level. Then come the extensive areas of the black grass, *Juncus Gerardi*, upon which, in part, the economic value of the marsh depends. Sometimes there are extensive areas covered with lesser salt grass, *Distichlis spicata*. The samphires, *Salicornia ambigua*, *S. europæa*, grow in pure associations, sometimes mingling with the lesser salt grass, *Distichlis spicata*. The sea lavender, *Limonium carolinianum*, is also found with the samphires, as also *Suæda maritima* and *Atriplex patula*. Finally fresh-water conditions begin to prevail and typical fen vegetation becomes dominant the marsh surface over.

Fen Formation.—The acquaintance of the writer with the vegetation of the Hackensack fenland is based on numerous trips across it by railroad between Newark and Jersey City, Hoboken and Rutherford, by several trunk lines, viz., the Pennsylvania, Baltimore and Ohio, Delaware, Lackawanna and Western, and the Erie at all seasons of the year. A detailed study of the marsh was made in company with Vincent G. Burns, who continued its study by a notable collection of plants during several growing seasons. The three most prominent associations of the fen formation are characterized by the dominance of one of three plants, *Phragmites communis*, *Typha latifolia* (inland), *T. angustifolia* (influenced by brackish water), and *Zizania palustris*.

Phragmites communis covers extensive areas and is impressive at all seasons of the year (Figs. 5, 6). In the spring its fresh, light greens are noticeable; in autumn and early winter its purplish plumes of spikelets bending gracefully with the wind are striking. The movement of the leaves by a turning of the sheaths through an angle of 180 degrees brings them all on to the leeward side of the stem, in the direction in which the wind blows. Where sand has been washed into the marsh the reed forms long running rhizomes, which, growing up out of the muck, stretch across the sand as leafy stolons, a measured distance, in one case, of 5.8 meters. Green, leafy, erect shoots

* *Harshberger, J. W.:* The Vegetation of the Salt Marshes and of the Salt and Fresh Water Ponds of the Northern Coast of New Jersey. Proc. Acad. Nat. Sci. of Phila., 1909: 373–400.

are produced all along this rhizome, which reaches the thickness of a lead-pencil.

Recently Harper,* in some dynamic studies of Long Island vegetation, has estimated that one acre of *Phragmites communis* about 10 feet tall, with 77 stems per square yard, or 372,680 stems per acre, on September 19th produced 48,400 pounds of fresh material, 24,000 pounds of air-dried material, and 1585 pounds of ash. In dry weight of the marsh plants *Phragmites* leads by a large margin.

Elsewhere the cattails, *Typha angustifolia* and *T. latifolia*, are supreme, the former growing most commonly where it is influenced by an occasional inundation of salt water, while the latter is more strictly confined to fresh-water conditions. These two plants compete with the reed grass, *Phragmites*, in the occupation of the marshland. It would be hard to say, without experimental data, just what conditions determine the success of one or the other associations of plants. It may be edaphic, or it may be purely historic, reasons which determine the nice adjustment of conditions which permit the growth of the cattails to the exclusion of the reed, and vice versa. Harper (*loc. cit.*) has shown that an acre of *Typha latifolia*, with 1 per cent. of other plants standing 5 feet tall, with 30 stems per square yard, produced 31,460 pounds of fresh material, 12,100 of dried material, and 296 pounds of ash. *Typha angustifolia*, in a fresh marsh at the head of a brackish marsh, growing 9 feet tall with 61 stems per square yard, all sterile, yielded 53,240 pounds of fresh substance, 15,443 pounds of dried material, and 843 pounds of ash. All these estimates are from plants growing in marshes on Long Island, east of New York City, and, therefore, the figures probably stand good for similar sized areas on the Hackensack marsh.

The wild rice, *Zizania palustris*, is found most usually in the deeper water along some stream or river controlled by fresh water, where it forms associations of considerable width and size. In early spring, as its shoots appear above the muck surface, they are light green, and as summer advances the plants sometimes grow to be 10 feet tall. In August, when the wild-rice fruits are ripe, the marsh is lively with various birds that feed upon the wild rice. Such are the reed birds (bobolinks) and Sora rail, also large flocks of red-winged blackbirds, while in July, August, and September swallows are by far the

* The Plant World, 21: 38–46.

FIG. 5.
Society of Reed Grass, *Phragmites communis*, in middle ground of picture, and *Hibiscus Moscheutos* in flower in the foreground. Typical expanse of Hackensack Marsh near the Penna. R. R. Trestle, August 31, 1916. V. G. B.

FIG. 6.
Reed Grass, *Phragmites communis*, near the Hackensack River Bridge on the Harrison Turnpike, August 25, 1916. V. G. B.

FIG. 7.
Reed Grass, *Phragmites communis*, surrounding a low tree of Wild Black Cherry, *Prunus serotina*, near a Branch of Sawmill Creek, August 15, 1916. V. G. B.

most abundant birds of the meadow and use the reeds as dormitories. These birds perch in long lines on roadside telegraph wires above the marsh.*

Associated with these three plants, varying here and there in abundance, is the rose-mallow, *Hibiscus Moscheutos*, which glorifies the grassy stretches in August with numerous large rose-pink to white flowers. The arrow-leaf, *Sagittaria latifolia*, is found in standing water everywhere in the area. *Scirpus cyperinus*, *Spartina cynosuroides* (Fig. 10), are abundantly associated with the taller plants, forming the same layer of growth. The Turk's-cap lily, *Lilium superbum*, noted by me in full flower on August 6, 1916, is not uncommon. The three-seeded mercury, *Acalypha virginica*, is a rare fen species, but *Impatiens biflora*, the spotted touch-me-not, is extremely common in shady places. Three members of the carrot family, UMBELLIFERÆ, seem at home in the wet muck, viz., the water hemlock, *Cicuta maculata*, *Sium cicutæfolium*, and the low *Ptilimnium capillaceum*, which seems to increase as the brackish part of the marsh is approached. The Indian hemp, *Apocynum cannabinum*, is common, along with the showy swamp milkweed, *Asclepias incarnata* var. *pulchra*, visited by thousands of butterflies. The hedge bindweed, *Convolvulus sepium*, climbs up the tall grasses and cattails, while the dodder, *Cuscuta compacta*, is parasitic on goldenrod. Two species of *Lycopus* (see list) are found in the marsh. The Hackensack fen is brightened in early September by the flowers of *Bidens lævis*, massed in bright golden patches, which, with the whites and pinks of the rose-mallow, *Hibiscus Moscheutos* (Fig. 9), and other plants with conspicuous colors (asters, goldenrod), turn the fenland into a wonderful flower-garden.

The ferns of the marshy swales are *Osmunda regalis* (Fig. 11), *Onoclea sensibilis*, *Aspidium Thelypteris*. The water of some of the drainage ditches is covered with floating duckweed, *Lemna minor* (Fig. 8), and several species of *Potamogeton* occur. The bladderwort, *Utricularia intermedia* (Fig. 12), filled a ditch with its bright yellow flowers on July 15, 1916.

Other taller grasses associated with the dominant ones are *Calamagrostis canadensis*, *Echinochloa Walteri*, and *Spartina cynosuroides* (in fresh-water marsh) (Fig. 10).

CARR FORMATION.—The woody plants of the Hackensack fenland are

* *McAtee, W. L.:* Three Important Duck Foods. Circular No. 81, Bureau of Biological Survey, Sept. 9, 1911: Five Important Wild Duck Foods, Bulletin 58, U. S. Dept. of Agri.; Eleven Important Wild Duck Foods, Bulletin 205, U. S. Dept. of Agri., May 20, 1915.

either isolated individuals, or else they are found in associations forming the marsh thickets, or carr. *Quercus bicolor*, the swamp white oak, is found in low swales bordering the fens, along with *Amelanchier oblongifolia*, *Pyrus arbutifolia*, *Rosa carolina*, *Sambucus canadensis*, and *Acer rubrum*. The wild cherry, *Prunus serotina*, grows entirely isolated in a *Phragmites* marsh, or it enters the thickets as a rather prominent element (Fig. 7). The Virginia creeper, *Psedera quinquefolia*, and pigeon grape, *Vitis æstivalis*, grow in the carr and clamber over the trees and shrubs, binding them together. The white ash, *Fraxinus americana*, is an element of moist thickets, as also the black haw, *Viburnum prunifolium*. These isolated shrubs and trees and the above thickets break the monotonous sky-line. In one or two places along the eastern edge of the marsh the thicket interspaces are invaded by trees and shrubs, so that in a few years, assisted by the artificial drainage which is taking place, the thicket formation will be extended so as to cover large areas of the open fen.

Vegetation of Snake Hills.—One other unit of vegetation should be mentioned in closing this account of the formation of the Hackensack Marsh. As previously mentioned by the junior author, Burns, there are two basaltic outcrops, represented by Snake Hill and Little Snake Hill, which support a dry upland vegetation. As they form conspicuous landmarks in the center of the marsh, actually dividing it into an upper and a lower portion, a brief account of the vegetation is appropriate. The trees of the Snake Hills are *Juglans cinerea*, *Betula lutea*, *Quercus Prinus* (abundant), *Celtis occidentalis* in rocky areas, *Sassafras variifolium*, *Hamamelis virginiana*, *Liquidambar Styraciflua*, *Acer saccharum*, *Cornus paniculata*, *Viburnum pubescens*, while the late blueberry, *Vaccinium vacillans*, forms the common undergrowth layer on these rocky hills. The herbaceous plants of such rocky hills are given in the list of plants collected by Vincent G. Burns and need not be mentioned here.

THE FLORA (V. G. B.)

In view of the fact that the difference between low and high tide levels in the Hackensack River is only 2 or 3 feet, and as one goes up the river the water becomes less salt, it would be expected that the flora would change from a typical salt marsh near Newark Bay, at the river mouth, to a brackish flora in the center of the valley, and finally to a fresh-water flora in the northern part. Also it would be expected that, as one went back from the river on

FIG. 8.
A stagnant pool covered with Duckweed, *Lemna minor*, and surrounded with Cattails, *Typha*, near the Belleville Turnpike, August 18, 1916. V. G. B.

FIG. 9.
Marsh Rose Mallow, *Hibiscus Moscheutos*, not far from Schuyler's Corner and the Belleville Turnpike, August 15, 1916. V. G. B.

FIG. 10.
Spartina cynosuroides in flower near the Harrison Turnpike, September 3, 1917. V. G. B.

either side the flora would become less and less that of the typical salt marsh. In a general way these conditions prevail. For example, *Spartina glabra* var. *pilosa* is very common along the lower banks of the Hackensack, but it is entirely absent above Sawmill Creek. Similarly, *Juncus Gerardi* and *Spartina patens*, typical salt-marsh plants, are found near Newark Bay and on the flats around Penhorn Creek, but there is not a sign of these plants in the upper part of the valley. In the northern part of the Hackensack region there are acid swamps, characterized by such oxylophytes as *Vaccinium corymbosum*, *Rhododendron viscosum*, *Acer rubrum*, and *Clethra alnifolia*. In the southern part acid swamps are absent, and it is interesting to note that the transition area between acid swamps and alkaline salt marsh is easily demarcated. In fact, one can draw an exact line of tension between salt and fresh-water marsh floras, since the latter is distinguished from the former by the absence of the characteristic grasses and grass-like plants. The following is a list of the plants collected and identified, with a note of the habitat and other matters of interest:

LIST OF PLANTS

DIVISION II.—PTERIDOPHYTA

Polypodiaceae

Aspidium Thelypteris (L.) Sw. (Wood Fern).

This fern is quite common throughout the marshes in shaded brackish situations.

Onoclea sensibilis L. (Sensitive Fern).

Moist brackish meadows and along ditches.

Osmunda regalis L. (Flowering Fern) (Fig. 11).

In ditches along Belleville Road.

Equisetaceae

Equisetum arvense L. (Common Horsetail).

Roadsides and gravelly banks. This plant seems to delight in soil which lacks humus.

Lycopodiaceae

Lycopodium alopecuroides L.

Swamps near Moonachie.

DIVISION III.—SPERMATOPHYTA

Class 1.—Monocotyledoneae

Typhaceae

Typha angustifolia L. (Cattail).

One of the most common plants of the marshes, growing chiefly in brackish habitats.

Typha latifolia L. (Common Cattail) (Figs. 4, 8).

Growing chiefly in the inner fresh-water parts of the Hackensack fen country.

Alismaceae

Alisma Plantago-aquatica L.

Common in shallow ponds and ditches.

Sagittaria latifolia Willd.

Found in ditches and standing water everywhere on the marshes.

Graminaceae

Andropogon furcatus Muhl.

Dry banks on Little Snake Hill.

Andropogon scoparius Michx. (Beard Grass).

Dry, rocky ground on Little Snake Hill and elsewhere.

Avena sativa L. (Oats).

Roadsides.

Calamagrostis canadensis (Michx.) Beauv. (Blue-joint Grass).

Rocky thickets bordering the marsh around Little Snake Hill.

Dactylis glomerata L. (Orchard Grass).

Fields and roadsides.

Digitaria sanguinalis L. Scop. (Crab Grass).

Common along roadsides and embankments.

Distichlis spicata L. Greene (Spike Grass).

Common typical salt marshes.

Echinochloa Walteri (Pursh) Nash.

Brackish meadows and marsh borders.

Eleusine indica Gaertn. (Goose Grass).

Railroad embankments.

Eragrostis pilosa (L.) Beauv.

Railroad embankments. Common.

Hystrix patula Moench (Bottle-brush grass).

Moist thickets on Snake Hill.

Leersia virginica Willd. (White Grass).

Wet woods on Snake Hill.

FIG. 11.

Clump of Royal Fern, *Osmunda regalis*, in ditch along Belleville Road, across Hackensack Marsh, July 15, 1916. J. W. H.

FIG. 12.

Shallow channel filled with Bladderwort, *Utricularia intermedia*, in flower, and Great Water Dock, *Rumex Britannica*, along its edge, Hackensack Marsh, July 15, 1916. J. W. H.

Panicum capillare L. (Old-witch Grass).

Common on sandy ground and gravelly embankments.

Panicum clandestinum L.

On Snake Hill and adjacent fields.

Panicum dichotomiflorum Michx.

Common on railroad embankments and dry parts of the marshes.

Panicum huachucae Ash.

Open fields on Snake Hill.

Panicum virgatum L. (Switch Grass).

Common along Sawmill Creek and other dry parts of the true salt marshes.

Phragmites communis Trin. (Figs. 5, 6).

This is perhaps the most common plant on the marshes. It grows everywhere, in both brackish and fresh parts, spreading freely by long creeping root-stocks and attaining a height of 10 feet. The ribbon-shaped green leaves of this plant give the marsh its light greenish hue of mid-summer.

Setaria glauca (L.) Beauv. (Foxtail Grass).

Open ground in the less brackish parts of the marsh.

Sorghastrum nutans (L.) Nash (Indian Grass).

Dry gravelly banks of Little Snake Hill.

Spartina cynosuroides (L.) Roth. (Salt Reed Grass) (Fig. 10).

This quite common species grows on the higher parts of the marsh well back from the river.

Spartina glabra var. *pilosa* Merr. (Salt-marsh Grass).

Along the Hackensack River, from Sawmill Creek southward.

Spartina patens (Ait) Muhl.

Occurs on the salt-marsh flats in great abundance, usually well back from the river.

Spartina patens var. *juncea* (Michx) Hitchc.

In brackish habitats and farther inland from the river than the species.

Zizania palustris L. (Indian Rice, Water Oats).

Mostly occurs along ditch borders in the northeastern corner of the valley. A gigantic grass with great stout culms and tall panicles, sometimes exceeding 10 feet. The natives call it "Bobolink Seed," because of the fondness of these birds for the grain.

CYPERACEAE

Cyperus filiculmis Vahl.

Dry sterile soil on Snake Hill.

Cyperus Nuttallii Eddy.

Common in brackish soil.

Cyperus strigosus L.

Damp soil along ditches, etc.

Scirpus americanus Pers. (Three Square).

Salt-marsh borders. At many places on the higher parts of the marshes this tall-growing sedge is cut and used for hay.

Scirpus cyperinus (L.) Kunth (Wool Grass).

Wet meadows and thickets bordering the marshes.

Stenophyllus capillaris (L.) Britton.

Dry sterile soil on Snake Hill.

ARACEAE

Peltandra virginica (L.) Kunth (Green Arrow Arum).

Shallow water of ditches.

LEMNACEAE

Lemna minor L. (Duck-weed) (Fig. 8).

On the surfaces of stagnant water in pools and ditches all over the marsh.

COMMELINACEAE

Commelina communis L. (Day-flower).

A frequent plant in low swales and ditches.

JUNCACEAE

Juncus canadensis J. Gay.

Occurring everywhere on the typical salt brackish marsh.

Juncus Gerardi Loisel (Black Grass).

This is one of the most typical salt-marsh plants and is found on all the true salt-marsh flats.

Juncus tenuis Willd.

Very common along roadsides and in dry fields.

LILIACEAE

Lilium superbum L. (Wild Yellow Lily).

Wet meadows and bogs near the marsh borders.

Lilium philadelphicum L. (Wood Lily).

Rare. Dry ground near Snake Hill.

Smilacina racemosa (L.) Desf. (False Spikenard).

Moist woods and banks on Snake Hill and Little Snake Hill.

Smilax rotundifolia L. (Common Green Briar).

Moist thickets near Snake Hill.

ORCHIDACEAE

Calopogon pulchellus (Sw.) R.Br.
Rich open grounds near Moonachie.
Habenaria ciliaris (L.) R.Br. (Yellow-fringed Orchid).
Meadows near Moonachie.
Pogonia ophioglossoides (L.) Ker.
Boggy meadows near Moonachie.

CLASS 2.—DICOTYLEDONEAE

SALICACEAE

Populus grandidentata Michx.
Rich thickets bordering the marshes.
Salix babylonica L. (Weeping Willow).
A few isolated trees on the marsh near Secaucus.
Salix cordata Muhl.
Wet places along Belleville Turnpike.
Salix nigra Marsh (Black Willow).
Wet banks near Snake Hill.

MYRICACEAE

Myrica carolinensis Mill (Bayberry).
Gravelly railroad embankments.

JUGLANDACEAE

Juglans cinerea L. (Butternut).
On Snake Hill in open woods.

BETULACEAE

Betula lenta L. (Cherry Birch).
Open woods on Snake Hill.
Betula populifolia Marsh (Gray Birch).
Sterile soil along the Belleville Turnpike.

FAGACEAE

Quercus alba L. (White Oak).
Dryish fields near marsh borders.
Quercus bicolor Willd. (Swamp White Oak).
Low swales bordering marsh.
Quercus Prinus L. (Chestnut Oak).
Rocky banks on Snake Hill and Little Snake Hill.

Quercus velutina Lam. (Black Oak).
Dry gravelly embankments.

URTICACEAE

Celtis occidentalis L. (Sugarberry).
Rocky woods on Snake Hill.
Humulus Lupulus L. (Common Hop).
Moist banks and rubbish heaps in the northeast section of the marsh.
Ulmus americana L. (American Elm).
Moist banks along Snake Hill.

POLYGONACEAE

Polygonum arifolium L. (Halberd leaved Tear-thumb).
Common in low grounds, clambering over other plants.
Polygonum aviculare L.
Common everywhere in waste places and on railroad embankments.
Polygonum Convolvulus L. (Black Bindweed).
Railroad embankments and waste places.
Polygonum Hydropiper L. (Common Smartweed).
Moist grounds.
Polygonum hydropiperoides Michx. (Mild Water Pepper).
Wet places and shallow water in brackish habitats.
Polygonum lapathifolium L.
Common on moist banks and on brackish meadows.
Polygonum orientale L. (Prince's Feather).
Moist ditches and waste ground.
Polygonum pennsylvanicum L.
Common in wet open meadows.
Polygonum Persicaria L. (Lady's Thumb).
Common moist places.
Polygonum sagittatum L. (Arrow-leaved Tear Thumb).
Clambering over other plants in low swales.
Polygonum scandens L. (Climbing False Buckwheat).
Clambering on Typha and Phragmites.
Polygonum virginianum L.
Moist woods on Snake Hill.
Rumex Acetosella L. (Sheep Sorrel).
A common weed everywhere on the dry places.
Rumex Britannica L. (Great Water Dock) (Fig. 12).
A tall, stout herb, growing in great abundance everywhere on the brackish flats.

CHENOPODIACEAE

Atriplex patula L.

Brackish flats on the Hackensack River banks.

Atriplex patula var. *hastata* (L.) Gray.

Hackensack River banks and inland.

Chenopodium album L. (Pigweed).

Everywhere on drier parts.

Chenopodium ambrosioides L. (Mexican Tea).

Waste places and brackish meadows.

AMARANTHACEAE

Acnida cannabina L.

A very tall, tree-like herb growing throughout the marshes in brackish stations.

Amaranthus retroflexus L. (Pigweed).

Roadsides and cultivated grounds.

PHYTOLACCACEAE

Phytolacca decandra L. (Pokeweed).

Gravel of railroad embankments.

CARYOPHYLLACEAE

Saponaria officinalis L. (Bouncing Bet).

Very common along roadsides, embankments, and in waste grounds.

Silene stellata (L.) Ait. f. (Starry Campion).

Woody banks on Snake Hill.

RANUNCULACEAE

Thalictrum polygamum Muhl.

Wet meadows near Belleville Turnpike.

LAURACEAE

Sassafras variifolium (Salisb.) Ktze.

Open woods on Snake Hill.

CRUCIFERAE

Lepidium virginicum L. (Wild Pepper Grass).

Common in waste places.

Sisymbrium altissimum L. (Tumble Mustard).

Roadsides and waste places.

SAXIFRAGACEAE

Parnassia caroliniana Michx.
Swamps near Moonachie.

HAMAMELIDACEAE

Hamamelis virginiana L. (Witch-hazel).
Open woods on Snake Hill.
Liquidambar Styraciflua L. (Sweet Gum).
Open woods on Snake Hill.

ROSACEAE

Amelanchier oblongifolia (T. & G.) Roem.
Moist thickets near Belleville Turnpike.
Potentilla canadensis var. *simplex* (Michx.) T. & G.
Dry gravelly soil along embankments.
Potentilla monspeliensis L.
Dry banks. Common.
Potentilla primula Poir.
Common on dry open soil.
Prunus serotina Ehrh. (Wild Cherry) (Figs. 7, 13).
Very common in every section of the marshes. Especially frequent along the banks of Sawmill Creek, where it is the only tree.
Pyrus arbutifolia (L.) L. f. (Chokeberry).
Swamps and low thickets.
Pyrus melanocarpa (Michx.) Willd.
Moist woods.
Rosa humilis Marsh.
Dry embankments along the Belleville Turnpike.
Sanguisorba canadensis L. (Canadian Burnet).
Bogs and wet swales. Common.
Spiraea tomentosa L. (Hardhack).
Wet banks along Belleville Turnpike.

LEGUMINOSAE

Desmodium canadense L. (Tick Trefoil).
Wet meadows and swales.
Gleditsia triacanthos L. (Honey Locust).
Railroad embankments, Delaware, Lackawanna & Western R. R., near Hackensack River.
Lespedeza frutescens L. (Bush Clover).
Sterile soil on Little Snake Hill.

FIG. 13.
Wild Black Cherry, *Prunus serotina*, forming with other shrubs a Marsh Thicket (Carr) on the bank of Sawmill Creek, August 15, 1916. V. G. B.

FIG. 14.
Shelter constructed of uprights and thatched with cut stalks of Reed, *Phragmites communis*, along Belleville Road, Hackensack Marsh, July 15, 1916. J. W. H.

Lespedeza hirta (L.) Hornem.
Sterile soil on Little Snake Hill.
Medicago sativa L. (Alfalfa).
Roadsides and railroad embankments.
Melilotus alba Desr. (White Melilot).
Roadsides. Common.
Melilotus officinalis (L.) Lam. (Yellow Melilot).
Roadsides. Common.
Trifolium arvense L. (Rabbitfoot Clover).
Dry gravelly roadsides.
Trifolium pratense L. (Red Clover).
Common in fields.
Trifolium procumbens L. (Low Hop Clover).
Sandy grounds and roadsides.
Trifolium repens L. (White Clover).
Common by roadsides and paths.

SIMARUBACEAE

Ailanthus glandulosa Desf. (Tree of Heaven).
Thickets along banks near Arlington.

EUPHORBIACEAE

Acalypha virginica L. (Three-seeded Mercury).
Belleville Turnpike Embankment. Rare.
Euphorbia maculata L. (Milk Purslane).
Makes great mats of dull red foliage on gravelly railroad embankments.
Euphorbia Preslii Guss.
Belleville Turnpike embankment. Rare.

ANACARDIACEAE

Rhus glabra L. (Smooth Sumac).
Dry banks and meadows. Common.
Rhus Toxicodendron L. (Poison Ivy).
Very common over rock and along embankments.
Rhus typhina L. (Staghorn Sumac).
Little Snake Hill.

CELASTRACEAE

Celastrus scandens L. (Climbing Bitter Sweet).
Common on rocky banks of Little Snake Hill.

ACERACEAE

Acer rubrum L. (Swamp Maple).

Very common in the acid swamps of the southern part of the valley.

Acer saccharinum L. (Silver Maple).

Rocky banks of Snake Hill.

Acer saccharum Marsh (Sugar Maple).

Woods all over Snake Hill.

BALSAMINACEAE

Impatiens biflora Walt. (Spotted Touch-me-not).

Abundant in shady moist places.

VITACEAE

Psedera quinquefolia L. Greene (Virginia Creeper).

Trailing over pipe lines which cross the marshes.

Vitis aestivalis Michx. (Pigeon Grape).

Thickets on the steep western slopes of Snake Hill.

MALVACEAE

Hibiscus Moscheutos L. (Swamp Rose Mallow) (Figs. 5, 9).

On brackish flats, borders of thickets, in shady moist copses, or out in the meadow. This is one of the most beautiful and striking plants in our wild flora. In August the marsh looks like a vast flower garden, for many areas are colored white and pink by the profusion of the large flowers.

ONAGRACEAE

Epilobium angustifolium L. (Fire-weed).

Gravelly embankments and roadsides.

Epilobium hirsutum L.

Rather rare on the marsh, having been seen by the writer only in an isolated spot near the Public Service Works.

Ludvigia alternifolia L. (Seed-box).

Banks of Little Snake Hill.

Oenothera biennis L. (Evening Primrose).

Very common in waste places.

UMBELLIFERAE

Cicuta maculata L. (Water Hemlock).

Common in wet meadows.

Daucus Carota L. (Wild Carrot).

Common along roadsides.

Ptilimnium capillaceum (Michx.) Raf.
Brackish marshland everywhere.
Sium cicutæfolium Schrank.
Common in moist situations.

CORNACEAE

Cornus Amomum Hill (Kinnikinnick).
Rare. One station only, along Belleville Turnpike, near Erie Railroad.
Cornus paniculata L'Her.
Thicket on summit of Snake Hill.

ERICACEAE

Clethra alnifolia L. (Sweet Pepperbush).
Between Secaucus and the Susquehanna Railroad.
Rhododendron viscosum (L.) Torr (Clammy Azalea).
In the swamp between Secaucus and the Susquehanna Railroad.
Vaccinium corymbosum L. (High-bush Blueberry).
In the swamp between Secaucus and the Susquehanna Railroad.
Vaccinium vacillans Kalm (Late Low Blueberry).
Common all over Snake Hill.

PRIMULACEAE

Samolus floribundus H. B. K. (Water Pimpernel).
Wet low places in the northern marshes.

OLEACEAE

Fraxinus americana L. (White Ash).
Moist thickets.

GENTIANACEAE

Menyanthes trifoliata L. (Buckbean).
Bogs and shallow water near Moonachie.
Sabatia dodecandra (L.) B. S. P.
Swamp near Moonachie.
Sabatia stellaris Pursh.
Salt marshes and around Little Snake Hill and Snake Hill.

APOCYNACEAE

Apocynum cannabinum L. (Indian Hemp).
Wet copses near the Belleville Turnpike and sandy banks elsewhere over the marsh.

Asclepias incarnata var. *pulchra* (Ehrh.) Pers. (Swamp Milkweed).
Common in swampy situations.

CONVOLVULACEAE

Convolvulus arvensis L. (Field Bindweed).
Waste places and railroad embankments.
Convolvulus sepium L. (Hedge Bindweed).
Clambering over tall herbs in marsh thickets.
Cuscuta compacta Juss. (Dodder).
Clambering over Goldenrods in moist thickets.
Cuscuta Gronovii Willd. (Dodder).
In wet shady copses, climbing on other plants.

BORAGINACEAE

Echium vulgare L. (Viper's Bugloss).
Common along roadsides.

VERBENACEAE

Verbena hastata L. (Blue Vervain).
Damp banks and roadsides.

LABIATAE

Collinsonia canadensis L. (Horse Balm).
Rich moist woods on western slopes of Snake Hill.
Lycopus americanus Muhl.
Damp soil near embankments.
Lycopus virginicus L. (Bugle Weed).
Rich moist grounds.
Nepeta Cataria L. (Catnip).
In gravel near the Harrison Turnpike.
Pycnanthemum virginianum (L.) Durand and Jackson.
Dry open hillsides of Little Snake Hill.
Teucrium canadense var. *littorale* (Bicknell) Fernald.
Common marsh thickets and low grounds.

SOLANACEAE

Datura Stramonium L. (Jimson Weed).
Common on rubbish heaps and gravel embankments.
Solanum Dulcamara L. (Bittersweet).
Northern part of the Hackensack region.

SCROPHULARIACEAE

Gerardia purpurea L. (Purple Gerardia).
Low marshland around Little Snake Hill.
Linaria vulgaris Hill (Toadflax).
Roadsides and fields.
Scrophularia leporella Bicknell.
Rich open woods on Snake Hill.
Verbascum Blattaria var. *albiflorum* Ktze. (Moth Mullein).
Common along roadsides.
Verbascum Thapsus L. (Comon Mullein).
Fields and gravelly banks.

LENTIBULARIACEAE

Utricularia intermedia Hayne (Bladderwort) (Fig. 12).
In a low ditch by the Belleville Turnpike.

PLANTAGINACEAE

Plantago lanceolata L. (English Plantain).
Common along roadsides and in fields.
Plantago major L. (Common Plantain).
Very common in all waste places.

CAPRIFOLIACEAE

Sambucus canadensis L. (Common Elder).
Very common in the drier parts of the marsh.
Triosteum perfoliatum L. (Wild Coffee).
Open fields on Snake Hill.
Viburnum prunifolium L. (Black Haw).
Moist thickets near Schuyler's Corner.
Viburnum pubescens (Ait.) Pursh. (Downy Arrow-wood).
Rocky banks of Little Snake Hill.

CUCURBITACEAE

Sicyos angulatus L. (One-seeded Bur Cucumber).
Clambering over low herbs in the northeastern corner of the valley.

LOBELIACEAE

Lobelia siphilitica L. (Great Lobelia).
Open meadow near Schuyler's Corner.

COMPOSITAE

Achillea Millefolium L. (Yarrow).

Very common along roadsides.

Ambrosia artemisiifolia L. (Ragweed).

Very common along roadsides.

Ambrosia trifida L. (Great Ragweed).

Forms thick green patches along marsh borders and ditches.

Arctium minus Bernh.

Aster paniculatus Lam.

Hackensack marsh.

Aster paniculatus Lam. var. *bellidiflorus* (Willd.) Burgess.

Hackensack marsh.

Aster Tradescanti L.

Bidens frondosa L. (Beggar-ticks).

Common in wet grounds.

Bidens laevis (L.) B. S. P.

In brackish marsh over the valley. In early September this plant makes great golden patches on the marsh.

Bidens trichosperma (Michx.) Britton.

Marshes and ditches in the north.

Chrysanthemum leucanthemum L. (White Daisy).

Common along roadsides.

Cichorium Intybus L. (Chicory).

Roadsides and banks.

Cirsium lanceolatum (L.) Hill (Bull Thistle).

Gravelly embankments and roadsides.

Erechtites hieracifolia (L.) Raf. (Fireweed).

Moist meadows. Common.

Eupatorium perfoliatum L. (Boneset).

Common in low copses.

Eupatorium purpureum L. (Joe-Pye Weed).

Low moist meadows. Common.

Eupatorium sessilifolium L. (Upland Boneset).

Meadows on Snake Hill.

Galinsoga parviflora Cav.

Railroad embankments.

Helianthus annuus L. (Sunflower).

Waste ground along ditches near the Susquehanna Railroad in the northeastern part of the valley.

Helianthus divaricatus L.

Dry copses and banks.

Helianthus giganteus L.
Low thickets and marsh. Common.
Helianthus strumosus L.
Dry banks.
Iva oraria Bartlett.
A rather common shrub in brackish places.
Lactuca scariola L. (Prickly Lettuce).
Very common along roadsides and railroad embankments.
Lactuca spicata (Lam.) Hitchc.
Low grounds. Common.
Pluchea camphorata (L.) D.C. (Salt Marsh Fleabane).
Common on the typical salt marsh.
Prenanthes alba L. (Rattlesnake-root).
Prominent in low marshy swales.
Prenanthes trifoliolata (Cass) Fernald (Gall-of-the-Earth).
Thickets on Snake Hill.
Rudbeckia laciniata L. (Cone flower).
Low moist meadows. Rather uncommon.
Solidago altissima L.
A common plant in rich open soil.
Solidago bicolor L. (White-flowered Golden Rod).
Common on dry soil on Snake Hill and Little Snake Hill.
Solidago canadensis L. (Common Golden Rod).
In thickets and rich open soil.
Solidago graminifolia (L.) Salisb.
Common in moist places.
Solidago neglecta Torr. & Gray.
Hackensack marsh.
Solidago nemoralis Ait.
Only on dry open places on Snake and Little Snake Hill.
Solidago rigida L.
Only on dry rock hillsides of Little Snake Hill.
Solidago rugosa Mill. (Rough-leaved Golden Rod).
Damp swales and wet thickets near roadsides.
Solidago sempervirens L. (Seaside Golden Rod).
Common on true salt marsh and on brackish flats back from the river.
Solidago ulmifolia Muhl.
Dry rocky woods on Snake Hill.
Sonchus asper (L.) Hill (Spring-leaved Sow-thistle).
Common along roadsides and embankments.

Taraxacum officinale Weber (Common Dandelion).
Abundant in waste places.
Tussilago Farfara L. (Coltsfoot).
On railroad embankments.
Vernonia noveboracensis Willd. (Ironweed).
In low swampy places. Common.
Xanthium commune Britt. (Cocklebur).
Shores of the Hackensack River.

ADDITIONAL LIST OF HACKENSACK MARSH PLANTS (J. W. H.)

The following additional names have been taken from the "Catalogue of Plants found in New Jersey," by N. L. Britton, Final Report of the State Geologist, II, 1889:

Polypodiaceae

Woodwardia virginica (L.) Smith (Chain Fern).

Pinaceae

Chamaecyparis thyoides (L.) B. S. P. (White Cedar).
A few trees on the meadow near Newark, according to W. M. Wolfe.

Najadaceae

Potamogeton amplifolius Tuck.
Hackensack River. Austin.
Potamogeton pectinatus L.
Hackensack River. Austin.
Potamogeton Robbinsii Oakes.
Hackensack River. Austin.

Cyperaceae

Carex trisperma Dewey.
Secaucus. Leggett.
Eleocharis olivacea Torr.
Abundant on Hackensack marsh. Leggett.
Eleocharis rostellata Torr.
Abundant on Hackensack marsh. Leggett.
Scirpus atrovirens Muhl.
Common on Newark marsh. Leggett.
Scleria triglomerata Michx.
Newark marsh. Le Conte.

Scleria verticillata Muhl.
Hackensack marsh, 1863. J. F. Allen.

ARACEAE

Calla palustris L. (Water Arum).
Hackensack marsh. G. C. Woolson.

IRIDACEAE

Heteranthera dubia (Jacq.) Mac. M. (Mud Plantain).
Common along the Hackensack. Austin.
Iris prismatica Pursh. (Slender Blue Flag).
Hackensack marsh. Leggett.

ORCHIDACEAE

Arethusa bulbosa L.
Hackensack marsh. G. C. Woolson.

RANUNCULACEAE

Coptis trifolia (L.) Salisb. (Gold Thread).
Swamps along Hackensack River. G. C. Woolson.
Ranunculus circinatus Sibth.
Hackensack River. Austin.

LEGUMINOSAE

Lathyrus palustris L. var. *myrtifolius* (Muhl.) Gray.
Hackensack marsh. Leggett.

POLYGALACEAE

Polygala cruciata L.
Hackensack marsh. Leggett; Base of Snake Hill—N. L. Britton.

ACERACEAE

Acer Negundo L. (Box Elder).
Banks of Hackensack River west of Closter. Austin.

LYTHRACEAE

Rotala ramosior (L.) Koehne.
Hackensack marsh. Torrey Catalogue, 1819.
Lythrum lineare L. (Loosestrife).
Hackensack marsh. Leggett.

SCROPHULARIACEAE

Limosella aquatica L. var. *tenuifolia* (Wolf) Pers.
Hackensack River. Austin.

CAPRIFOLIACEAE

Viburnum nudum L.
Hackensack marsh. Leggett.

COMPOSITAE

Liatris spicata (L.) Willd.
Hackensack marsh. W. M. Wolfe.
Solidago Elliottii Torr. & Gray.
Hackensack marsh. Carey.

ECONOMIC CONSIDERATIONS (V. G. B. AND J. W. H.)

Many experiments have been tried with *Phragmites* and other grasses and sedges which grow on the marsh with a view to their utilization in the making of cord and twine and in the making of paper pulp for the manufacturing of paper. Recently, however, a chemical process has been perfected by which marsh grasses and sedges become available for this purpose. Thousands of tons of marsh grasses and sedges are rotting each year on the Hackensack meadow that might be used profitably in the manufacture of paper, bagging, and roofing felts. Figure 14 illustrates the use of the marsh plants for shelter construction.

It is a well-known fact that reclaimed salt marsh makes good agricultural land. The reclaimed salt marshes of Holland, Belgium, and Nova Scotia are excellent examples of the value of such land. There the farmers have built dikes around the marshes to keep out the sea, and at intervals of several years the tide is allowed to flood limited portions of the polderland. This flooding replenishes the stock of certain salts in the soil without actually causing a return to the earlier conditions, if the flooding is properly regulated. According to W. F. Ganong, the same process has been used around the Bay of Fundy, where some of the largest and finest hay crops in eastern Canada have been produced.

These successes elsewhere suggest that the soil of the Hackensack marsh, if reclaimed, would become equally valuable. A system of dikes and sluice gates, perhaps, would be the most effective way of bringing about the desired

result. The fresh water, coming down the rivers and creeks from the interior, could pass out to sea through the sluiceways provided for this purpose, while the mechanism of the gate would prevent the salt water at high tide from flooding the agricultural land. Such a method would lower the water-table throughout the protected marsh, and by a system of drainage ditches the salts deposited originally by the sea water would be leached out of the soil by the rain water.

In the case of the Hackensack marsh, drainage ditches have helped considerably in the amelioration of the wild marsh conditions. This land has become extremely valuable for railroad terminals and factory sites, as much as $4000 per acre having been paid for some of it. The factory buildings have been built on foundations of concrete laid on the top of wooden piles, and the experience of the engineer in the rebuilding of the Venetian Campanile shows that such piles, if buried in the muck, may last many hundred years. The Campanile of San Marco was begun before the year 997 A.D. After its collapse, July 14, 1902, the foundations were studied by Sig. Giacomo Boni. The piles of white poplar were 9½ inches in diameter, driven into a bed of compact clay. When these were laid bare during the excavation preceding the reconstruction of the tower, the white poplar piles were found to be remarkably sound, retaining their color and fibrous character. This points to the conclusion that the men who build factories on timber piles in the Hackensack marshes, provided the piles are driven properly, need not fear a decay of the piles as long as the air is kept from them, which promotes the growth of bacteria and timber-destroying fungi. This fact is also emphasized by the discovery of undecayed stumps of white cedar trees in situ in certain parts of the marsh, as previously described. Such construction has been found to be costly on account of the depths to which the piles must be driven to give a secure foundation. Pile driving with a reclamation of the marsh land by scientific drainage will solve the difficulty, for D. C. Willoughby, a British engineer who has been working for years in building union railroad terminals on the Hackensack marsh, has come to the conclusion that before the marshes can be extensively used for building operations they must be thoroughly drained.

TRANSACTIONS OF THE WAGNER FREE INSTITUTE OF SCIENCE

OF

PHILADELPHIA

VOL. IX—PART 2

MARCH, 1921

WAGNER FREE INSTITUTE OF SCIENCE
MONTGOMERY AVE. AND SEVENTEENTH ST.
PHILADELPHIA

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ON THE LIFE HISTORY OF AN ECONOMIC CUTTLEFISH OF JAPAN, OMMASTREPHES SLOANI PACIFICUS

By MADOKA SASAKI

The Hokkaidô Imperial University, Sapporo, Japan

(With nine figures)

INTRODUCTORY

AMONG the Japanese marketable cuttlefishes the species of the most economic importance is *Ommastrephes sloani pacificus* Steenstrup, which is popularly known as "*Surumé-ika*" or "*Nibanzurumé.*" According to the statistical reports of the Agricultural and Commercial Department of the Japanese Imperial Government, the total annual catch of the species has often exceeded 70,000,000 kilos.

Rather suddenly there occurred a marked decrease of catch, as it took place in certain provinces some years ago. The condition is often repeated, so that villages which have been living upon this fishing industry suffer serious loss. The people of these localities are anxious to learn what causes this decrease, hence investigation is urgent. This condition caused me to undertake the present investigation at the request of one of the provinces which has suffered since some twelve years ago.

I have had several opportunities to visit the villages where cuttlefish catching is carried on, and to be on board fishing vessels. I spent the summer of the year 1919 in Sado, Oki, Tsushima, and Utsuryotô (Dagelet I.), islands scattered in the Japan Sea and the most famous places in all Japan for the cuttlefish industry and enjoyed special advantages for observing conditions. I have occupied myself in angling for the animal, in order to observe at the same time the physical conditions of the sea. The present paper embodies the results thus obtained except some parts, of which the investigation is still in progress to be published on a future occasion.*

* In this connection I wish to express my warmest thanks to Prof. S. Hatta and Dr. L. Balderston for their courtesies. My thanks are also due to Mr. Kitahara, leader of the Oceanographic Institute of the Agricultural and Commercial Bureau, Tokyo, for his kind advice and help in the course of the present investigation. Further, I am also indebted to Mr. S. Takarabé, Governor of Shimané-ken, and Vice-governor K. Iwamoto for their assistance in my work.

Besides the life history of the species, this paper deals with the morphology of the seminal receptacle, and the results of artificial fertilization of the eggs, as well as certain facts of the hydrography around the Japanese Islands, so far as these have important bearings on the present investigation.

DISTRIBUTION

According to the statistical reports referred to, the fishing places for *Nibanzurumé* appear to extend throughout the coasts of the Japanese Islands from Hokkaidô on the north to Loochoo on the south. It must, however, be borne in mind that the name *Nibanzurumé* is so vaguely used that it does not always indicate the species above referred to, but implies different species. In Loochoo, for instance, the name is applied to *Symplectoteuthis ouolaniensis*, which is caught there in plenty for market. In the northern parts of Hokkaidô, furthermore, both *Onychoteuthis banksii* and *Ommastrephes sloani pacificus* are taken together into the statistics under the name of *Nibanzurumé*.

The statistics of the reports are, though incomplete, enough to give a fair idea as to the density of distribution of the cuttlefish. From the statistics I have estimated, in respect to each prefecture or even each district, the mean amount of the annual catch of the species from 1908 to 1917. In the accompanying map (fig. 1) I endeavored to show the mean annual catch obtained in this way by dottings in the definite number per unit area, so as to give the density of the coastal distribution. From the map it is clear that on the whole the species is much more abundant on the coast of the Japan Sea than on the Pacific side. In the former the animal seems to live not only along the coast, but also in the open sea, at least in summer, in which season it is reported that cargoboats bound between Vladivostock and Japan have made good catches on their way. Similar facts have been communicated by Mr. K. Kuroda, teacher of fishing in the Hokkaidô Imperial University, who caught the species on almost every part of the line between Hokkaidô and Vladivostock in September, 1915. On the contrary, on the Pacific side of the Japanese Islands the distribution is quite limited within a few miles off the coast, except along Aomori-ken and Iwaté-ken where it may extend some twenty miles in summer and autumn.

SEASONAL AND DAILY MIGRATION

In all the coasts which I have visited, it is said that every year schools or shoals of the cuttlefish come from south or west and go away to north or east as a migratory fish does. For instance, in Sado, it is said, the schools appear for the first time in the Noto Peninsula in the early part of May, reach the west coast of Sado in the middle part of the same month, and then come to its east coast towards the end of the month. Thereafter the schools turn northward to Hokkaidô. In Oki the people say that the schools are seen some ten days after their appearance in Utsuryotô, and then after a fortnight the fishing takes place around the Noto Peninsula. In Utsuryotô, fishermen say, the cuttlefish come over from Tsushima, while the people of the latter isle believe the home of the animal to be in Kiushiu. Taken together, the information from these sources points to the conclusion that the animal migrates northwards from Kiushiu as far as into Hokkaidô. In spite of my efforts, however, I could not gather reliable data to support this conclusion. It is true, so far as concerns the beginning of the fishing season, which grades in time from south or west to north or east. In fact the season begins, on the whole, earlier and ends later in the southern seas

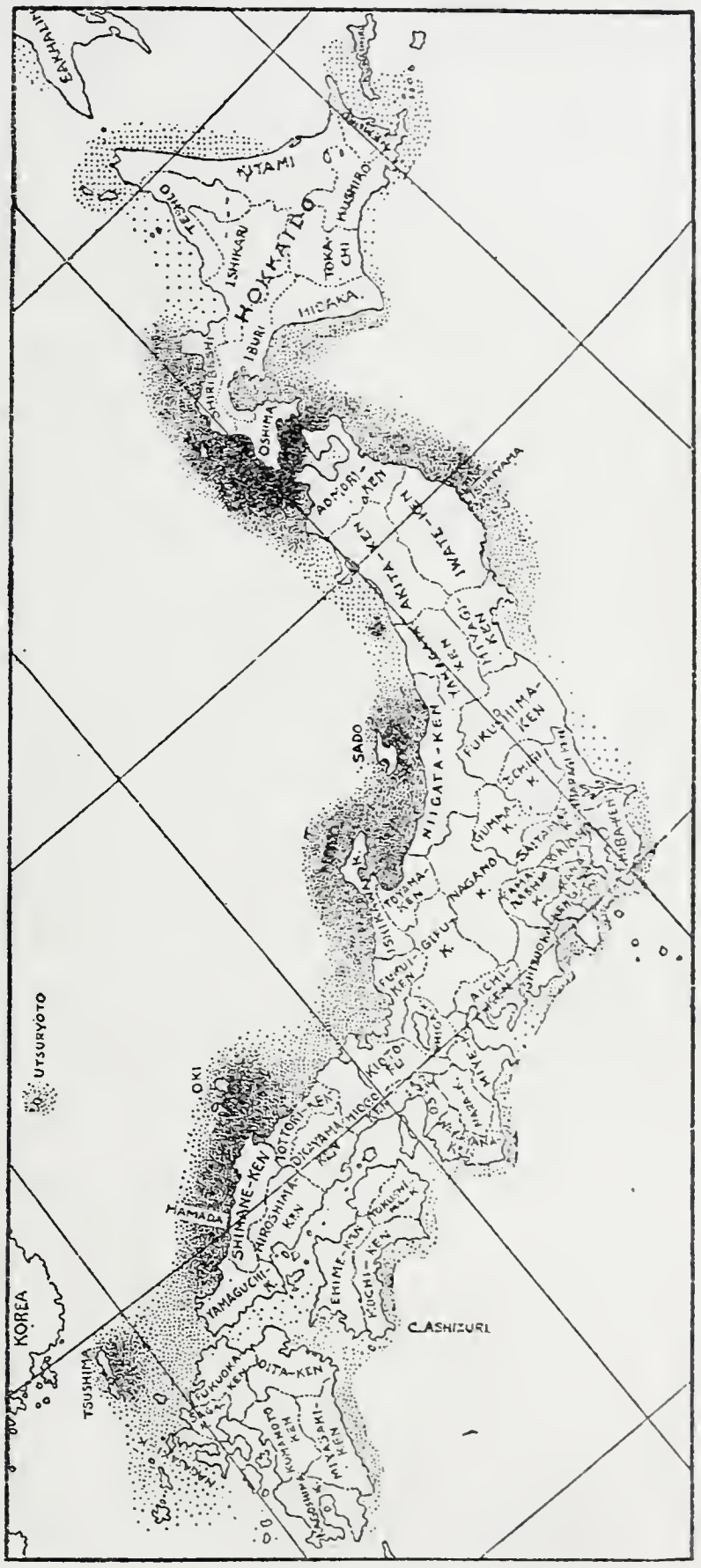

FIG. 1.

Map showing the density of distribution of *Nibanzurumé* (*Ommastrephes sloani pacificus*): compiled from the Statistical Reports of the Bureau of Agriculture and Commerce.

than in the northern part of Japan. In Hokkaidô the fishing is carried on usually from July to November, while in Oki its season continues all the year round except March and April, when the sea water there is coldest. The cuttlefish is usually most abundant everywhere in summer, so that sometimes it is called "*Natsu-ika*" (summer squid). The cuttlefish of the northern waters differs from that of the southern waters in the modes of the development and maturation of the reproductive organs as well as in certain characters of the habit, which will be considered later (pp. 13, 14). At all events these facts all tend to disprove the view of a long migration of the schools from Kiushiu to Hokkaidô.

The fishing is carried on daily from evening to morning, the best catch being obtained at somewhile after sunset and before sunrise. Even in daytime, by the use of suitable methods, one may catch the animal, though small in amount. Repeated experiences of angling show that in the daytime the cuttlefish are most abundant in the water strata from 50 fathoms to 100 fathoms, although such strata of the thickest population seem to differ in some degree in different seasons and localities. Numerous hydrographic observations made while fishing show us that the water temperature suited for the living of the cuttlefish is from 10° C. to 17° C., but that the animal of the northern seas is adapted to colder water than the southern form. From the facts above mentioned we may guess the daily migration of the cuttlefish to be as follows: They swim in daytime usually in the strata from 50 to 100 fathoms which are the part of the sea hardly penetrated by the daylight, as has been proved in oceanography. As the sun sets, they come up and are crowded together in the sea strata of about 20 fathoms or even near the surface, as if seeking a region of weak daylight. Towards midnight they again scatter themselves or sink, but are gathered once more near the surface before the sunrise. During these daily vertical migrations, however, the animal seems not much affected by the change of temperature, since this often varies greatly in the different water strata which it crosses.

Although there seems to be no special leader among a school, they are apt to move after a member which is excited; it is from this peculiar habit that one of the members angled up is followed by the remainder, which are thus easily brought up toward the surface; and when one is frightened by an enemy or by any other cause, all will at once disappear.

FOOD

The chief method of catching is by angling. The implements vary according to different localities, but the squid jig is the most universally used (fig. 2). The jig is pulled through the water, so that it resembles a rapidly swimming fish. This feature of the fishing stands in close relation to the natural food, on which the animal lives. I have examined the contents in the stomach of about 250 grown individuals from various localities. Of the contents examined, about 70 % consisted of fishes, 20 % of cuttlefishes, and the remaining 10 % of crustaceans and others. The species determination of these was of course by no means easy, because they were greatly mangled and so well digested that only their hard parts were left. The size of the food animals was, therefore, likewise not easy to be determined, but we may roughly infer it from the size of the jig which the animal likes to take, which varies, as is well known, from 60 mm. to 100 mm. in length. The fishermen, who are well aware of what size is best suited for the cuttlefish at a given period, use larger jigs as the fishing season proceeds. In some places fishermen use a larger anchor-shaped hook, through the stalk of which a grown mackerel is put as bait. When the cuttlefish are closely crowded, fishermen can draw up three or four of them together. This suggests that the cuttlefish may attack fishes larger than themselves.

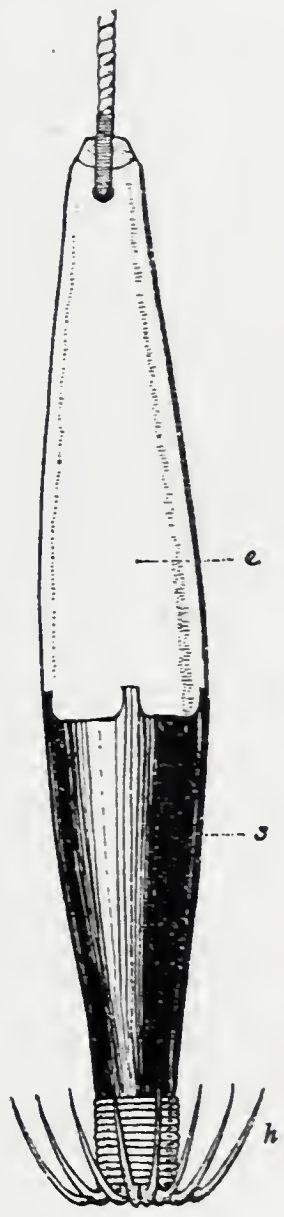

FIG. 2.
Squid jig to catch *Nibanzu-rumé*, used in Hokkaidô and Tsushima, × ⅔. *h*, Hook made of iron or brass; *e*, enticing piece made of bone or horn; *s*, sinking piece made of lead.

In younger individuals below 85 mm. in mantle length, the natural food is quite different from that of the adult so far as my examinations show. It consists of smaller floating organisms and among these microcrustaceans predominate.

RATE OF GROWTH

With the material so far examined it has not been possible to determine the exact age of maturation and the duration of life. The anatomical structures I have examined do not furnish any index of the age of the cuttlefish as do the scale and otolith of ordinary fishes, by means of which the annual rate of growth

may be estimated. A collection of specimens from Hokkaidô placed at my disposal provides, however, a key for estimation of the rate of growth, as seen in the following table, which is arranged according to the order of the months:

No.	*Number of specimens*	*Date of catch*	*Mantle length*
i	1	July 5, 1911	15.5–17.5 mm.
ii	5	July 15, 1910	170–194 "
iii	1	Aug. 30, 1912	195 "
iv	3	Oct. 15, 1912	190–215 "
v	8	Nov. 1, 1911	210–222 "
vi	2	Nov. 26, 1912	218–235 "
vii	2	Dec. 14, 1914	215–230 "
viii	7	Dec. 28, 1914	195–250 "

We see that the individuals caught in July are the smallest and those in December the largest, while gradual growth is going on in the intervening months, so that it is not difficult to find the rate of growth. This method of estimation could not be, however, extended and applied to the specimens from other localities, which could neither be obtained in so regular an order nor in so fresh a state. On the other hand, it has been found that the individuals grow heavier as the fishing season proceeds. The following is compiled from the returns from merchants of Sado and Tsushima who have long been dealing in the dried cuttlefish:

DRIED CUTTLEFISH FROM SADO

Average weight	*Month of catch*
ca. 15 grams	May and June
ca. 27 "	July and August
ca. 55 "	Sept. and Oct.
ca. 65 "	Nov. and Dec.

SAME FROM TSUSHIMA

ca. 10 grams	May
ca. 26 "	June and July
ca. 55 "	Aug. and Sept.
ca. 70 "	Oct. and Nov.

From the above table it is, therefore, also true that the animal is, in accordance with the estimation of the previous table, increasing in weight as the months proceed. This fact can not be proved to be true in some localities. In Toyama Bay, for instance, I got in the same month, April, specimens of every grade of age from very young ones to adult, and in Oki, on the contrary, there have been found grown individuals nearly all the year round. These

facts doubtless show that in these regions the spawning of the cuttlefish continues throughout nearly the whole year.

It is interesting that very young individuals with mantle length measuring only 60 mm. are in many places caught in plenty with fishing nets in March or April—a month in which, as seen from the tables above given, there is very little or no catch at all, for instance, in Hokkaidô, Sado, Tsushima, etc. It follows, therefore, that these individuals may be derived from spawning of the previous autumn or winter, and grow at the rate as above mentioned during the succeeding summer and autumn. It is further true that they may become mature in a year.

DEVELOPMENT OF MALE GENITAL ORGANS AND PAIRING SEASON

The hectocotylus is the left ventral arm, of which the full-formed structure has already been described by Dr. Ishikawa (1913).

The rate of its development varies to some extent in different localities. In Hokkaidô, as far as I have been able to ascertain, its transformation becomes discernible for the first time in the male of about 190 mm. mantle length, while Oki males of 176 mm. mantle length show fairly distinct hectocotylization, so that compared at about the same stage, the transformation of the arm is generally further advanced in the specimens from Oki than in those from Hokkaidô. Moreover, in Hokkaidô occurrence of full-formed hectocotylus is confined to December, which represents, in this locality, the final part of the fishing season, whereas in Oki, so far, hectocotylized males may be caught nearly all the year round. These facts as to the hectocotylization are parallel with the development of the internal genital organs, as given in the following tables:

Microscopic examinations show that the testis becomes mature when it attains a length of about 80 mm. As shown in the above tables, the testes of all the males from Oki are longer than this measurement and mature, and numerous spermatophores are found at any time of the year, while in Hokkaidô males provided with a testis so much developed are confined to December. It is thus evident that the maturation of the testis goes hand in hand with the hectocotylization so as to be ready for pairing. Such a seasonal contrast as between Oki and Hokkaidô, in respect to the maturity of the male genital

organs, is found also between other colder and warmer seas: in the latter, mature males are caught abundantly all the year round, with the exception of spring and early summer, during which seasons mature males are less numerous than immature ones.

SPECIMENS FROM HOKKAIDÔ

Date of catch	*Number of specimens examined*	*Length of mantle*	*Length of testis*	*Number of spermatophores in Needham's sac*
June 25	6	84–102 mm.	18–20 mm.	0
July 5	3	170–175 "	26–27 "	0
July 15	3	190–193 "	26–28 "	0
Aug. 30	2	194–195 "	27–28 "	0
Oct. 3	5	200–205 "	35–45 "	0
Nov. 1	5	210–220 "	48–55 "	0
Dec. 14	2	215–230 "	85–90 "	0–20
Dec. 28	3	195–215 "	82–95 "	0–20

SPECIMENS FROM OKI

Sept. 7, 1918	7	210–230 mm.	95–120 mm.	98–142
Oct. 21, "	5	205–230 "	90–102 "	67–212
Dec. 24, "	5	210–240 "	95–112 "	78–142
Jan. 24, 1919	6	220–233 "	90–110 "	76–183
Feb. 15, "	6	185–220 "	95–155 "	52–121
May 12, "	5	196–214 "	88–105 "	48–115
July 19, "	7	178–217 "	70–110 "	0–165
Aug. 3, "	4	200–210 "	96–102 "	70–130
Aug. 24, "	10	195–233 "	100–110 "	0–202

Now we see that the principal pairing season continues in warmer waters, for instance, in Oki, from summer to winter, while in colder waters, as in Hokkaidô, it is limited to the early part of winter.

Another evidence in regard to the pairing season is concerned with the spermatophores sticking in the buccal membrane of the female. Grown females have a number of spermatophores adhering to the buccal membrane, as in the Loliginidae and Sepiidae. Their adhesion would not occur unless the mating took place beforehand; this fact can, therefore, be regarded as an undisputable announcement of the pairing season of the cuttlefish and goes far to justify my assumption that the spawning season in Hokkaidô is confined to December, and that in Oki it extends from summer to winter.* It is hardly necessary to add that at the season mentioned the male reproductive organs are ripe.

* In Oki the season is continued into spring, as shown by catch of a small amount in this season.

The buccal membrane of the female has in its tissue about thirty seminal receptacles arranged in a circle (fig. 3). In the mated female the receptacles appear opaque white in color, being full of spermatozoa which have undoubtedly penetrated into the tissue, being set free from the spermatophores on the membrane. It is noteworthy that many females examined had an immature ovary, though they are provided with spermatozoa in their receptacles. The spermatozoa may, of course, remain alive in the receptacles. It follows that the pairing season does not necessarily exactly coincide with the spawning season. I may be permitted to add that the above mentioned position of the receptacles is not without significance but stands in an important connection with the spawning habit of the animal,* a subject to which I will return later (see pp. 13, 14).

FIG. 3.
Inner view of the buccal membrane of a mated female *Nibanzurumé*, nat. size. *bm*, Buccal membrane; *il*, inner lip; *ol*, outer lip; *sr*, seminal receptacle.

MATURATION OF FEMALE GENITAL ORGANS AND SPAWNING SEASON

The nidamental glands of the female make their first appearance in females of about 150 mm. mantle length and reach a size of about 20 mm. in those of about 200 mm. mantle length, while in fully mature females, which are above 280 mm. mantle length, the glands attain a length of about 180 mm. and a weight of about 60 grams, being quite as large and heavy as in the littoral cephalopods like the Loliginidae (fig. 4).

The ovary is still immature in the females below 200 mm. in mantle length. In the fully mature females referred to above, the ovary is about 48 grams in weight, occupying the posterior half of the mantle, and is filled up with the mature eggs only. The oviducts of these females, full of mature eggs, weigh about 30 grams each.

Fully mature females, such as above mentioned, are very rare. I have obtained them in the Bay of Toyama in spring and in Hokkaidô in summer.

* The Loliginidae and Sepiidae are provided with a pair of dendritic or racemose seminal receptacles in the tissue of the buccal membrane, in a similar way to that just mentioned, and deposit eggs fixed on the sea bottom or something found there.

The mature females ordinarily met with are not so fully matured, with their ovary less than half full of mature eggs and with the nidamental glands less than 120 mm. in length. The table gives, among various data, the sexual maturity of the female specimens which I have obtained from Oki.

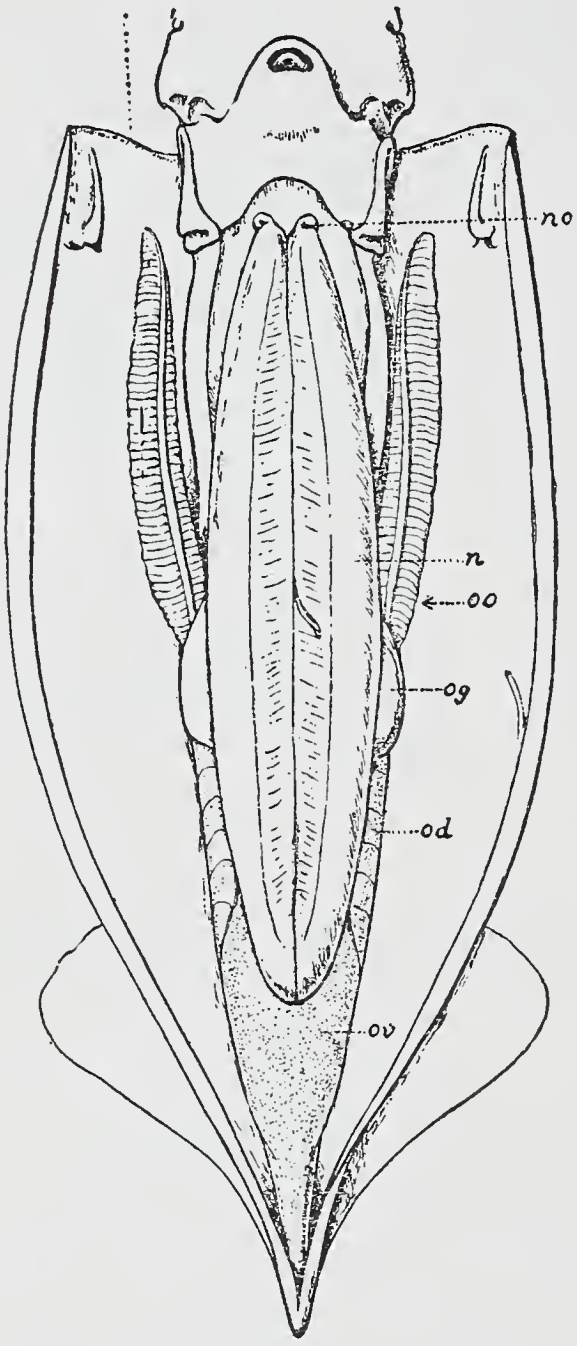

FIG. 4.

Mantle of a fully mature female *Nibanzurumé*, dissected along the midventral longitudinal line, × ⅓. *n*, Nidamental gland; *no*, external orifice of nidamental gland; *od*, oviduct; *og*, oviducal gland; *oo*, genital opening; *ov*, ovary.

From the table on p. 8, based upon anatomical examinations, we conclude that in Oki the principal spawning period confines itself to about September and October, even though the spawning continues all the year round, while the principal pairing period is extended from summer to winter; in short, the principal spawning period is much shorter than the principal pairing period. From the occurrence of the animal parallel data can also be collected. In nearly all warmer seas plankton feeding juveniles of the cuttlefish as caught with various kinds of fishing nets are most abundant synchronously with spring. In addition to this, the individuals caught at a certain season of the year are, on the whole, of approximately the same size. The gregarious occurrence of the same- or nearly same-aged individuals indicates a certain season in which they have been hatched out, and which represents, accordingly, a principal spawning period. On the other hand, in the collections taken all over the year there are found numerous young specimens measuring up only to 30 mm., proving that the spawning is taking place all the year round. As before mentioned, in the Bay of Toyama nearly all stages of growth were collected at a time and in Oki grown individuals have been caught at nearly all seasons. In other words, the spawning season continues throughout the year, just as we have pointed out from the anatomical side.

No.	*Date of catch*	*Number of specimens examined*	*Number of specimens sexually matured*	*Number of mated specimens*	*Length of nidamental gland*	*Length of mantle*
i	Sept. 7, 1918	3	3	3	65– 90 mm.	220–250 mm.
ii	Oct. 27, "	5	5	5	70– 95 "	210–248 "
iii	Dec. 24, "	1	0	1	50 "	252 "
iv	Jan. 24, 1919	2	1	2	48– 78 "	240 "
v	Feb. 15, "	4	0	4	35– 50 "	230–235 "
vi	April 12, "	5	1	0	..	198–232 "
vii	July 19, "	3	1	1	0–115 "	178–232 "
viii	Aug. 3, "	6	4	4	0–105 "	115–255 "
ix	Sept. 16, "	7	4	7	75–106 "	257–285 "
x	Sept. 27, "	9	6	9	79–121 "	230–279 "
xi	Oct. 14, "	9	8	9	91–121 "	255–288 "
xii	Dec. 26, "	9	1	8	0– 76 "	191–303 "
xiii	Jan. 5, 1920	3	1	3	60–106 "	260–303 "

MATURE EGGS AND THEIR INCUBATION IN THE NATURAL STATE

The mature eggs in the ovary or oviducts are easily distinguished from immature ones; the former are brown and transparent, while the latter are white and opaque. They are oval, measuring 0.6 mm. by 0.7 mm. The eggs deposited in the natural state have not yet been seen by naturalists nor known to any fishermen with whom I am acquainted. No exact datum on this point has been secured from the plankton investigations hitherto described, even though it has been suggested by observers that Oegopsid Cephalopods in general discharge eggs floating on the ocean. My efforts to prove them in the plankton collections made in Oki have also been in vain, although these collections represent every month in a region where mature females occur nearly all the year round. At first sight this seems very strange, bearing in mind that the species is among the commonest inhabitants in Japanese waters. To get light on this difficulty we have to turn to the anatomical details in relation to the nidamental glands and genital openings.

The nidamental glands are fused together and situated at the midventral part of the viscera. When mature they become so large as almost to cover the ventral surface of the visceral mass, and external orifices of the glands are found widely separated from those of the oviducts, in contrast, for instance, to *Watasenia* which is known as depositing pelagic eggs. Moreover, the latter genus has no seminal receptacles on the buccal membrane. The peculiar topographic relation of the genital openings to the nidamental orifices as seen

in the present species causes, therefore, the occurrence of the seminal receptacles on the buccal membrane which indicates the habit of depositing demarsal eggs. As I am convinced from these anatomical data, the present species can with reasonable certainty be said to discharge eggs which have not yet been observed, but will be detected in future imbedded in masses of a jelly-like substance deposited on the sea bottom or fixed to something on or near the bottom, at a depth accessible only by means of a proper dredging implement. On the other hand it has been made out by my experiments that the artificially fertilized eggs which have been reared so far as to be divided into segments, are denser than sea water. They must, therefore, be demarsal eggs, affording a strong positive evidence for my assumption just given.

ARTIFICIAL FERTILIZATION

In Oegopsid Cephalopods, as far as I have been able to ascertain, the results of artificial fertilization have not as yet been fully described on any species. In 1912 I attempted it with *Watasenia scintillans*, but was obliged to break off the experiment before it succeeded. Last summer I was so fortunate as to have a good opportunity of undertaking the experiment on *Ommastrephes sloani pacificus*, which was done twice. On the first occasion, on August 27, 1919, the material employed for the purpose consisted of one male and one female which were caught two miles off Saigo, Oki Islands. They were brought to me at 11 o'clock p. m., when they were still active. I took eggs out of the oviducts, and the spermatozoa from the spermatophores contained in Needham's sac of the male. The sexual elements were mixed together by the dry method and put into a basin containing sea water brought in from the offing. The eggs thus treated all sank to the bottom of the basin and did not float as in the case of *Watasenia* above referred to. They developed fairly rapidly, attaining the blastula stage by the next morning. The segmentation was partial and the cleavages extended on the yolk sphere nearly as far as in the case of *Loligo*. Towards noon the eggs began to die and by 3 o'clock p. m. all were dead and had become opaque.

In this experiment the eggs which developed were about 20% of the whole eggs treated, another 20% were dead shortly after the fertilization, and the remainder at last showed no sign of fertilization, although they remained as if living for some time.

The second experiment was performed on the succeeding day at about the same hour as the first. The material consisted, in this case, of one male and two females caught at the same part of the sea as in the last case, and unfortunately all the specimens were found dead twenty minutes before reaching my hand. The eggs I took out of the oviducts of the female as in the last experiment, but the spermatozoa were prepared from two different sources: firstly from the spermatophores of the buccal membrane of the females, and secondly from the spermatophores of Needham's sac of the male. Notwithstanding the experiment had been carried out as carefully as possible yet the rate of fertilization was much lower than the former experiment, the fertilized eggs being 10 % of the whole eggs treated with the spermatozoa from the first source and 5 % of those with the second. This low percentage of fertilization compared with the former occasion is undoubtedly due to the genital elements having been taken from dead individuals. The development was also not normal but mostly pathologic. Their final fates were the same as in the last.

Due to the bad condition of the sea, I had no opportunity for further experiment. At all events, however, from the two experiments referred to, I convinced myself that the artificial fertilization of the species in question is not difficult at all, wherever living material can be obtained, and that only one female is enough to afford the sexual elements for an experiment, and further that the early development does not widely differ from that of *Loligo*. Moreover, I call attention to the fact that the eggs remained on the bottom of the basin throughout the development so far examined, because this shows the eggs to be demarsal in the natural state also.

RELATION OF PHYSICAL CONDITION OF SEA TO HABIT OF NIBAN-SURUMÉ

The daily catch of the cuttlefish fluctuates very irregularly. This is, perhaps, largely due to the biologic characteristics of the cuttlefish relating to its food and enemies, as mentioned before (p. 4). These are, of course, combined with daily and seasonal changes of hydrographic and meteorologic conditions, which control the work of the fishermen. For instance, in autumn and winter the Japan Sea is usually so very rough that only the strongest and most skilful men can practise the fishery, and this, only in relatively quiet parts of the sea, sheltered from wind; this diminishes greatly the crop of the season,

even though the cuttlefish are as much crowded as in summer when the sea is usually quiet.

The above fluctuations of the crop are, however, a rather usual occurrence. Since some ten years ago, cases of great decrease of the catch have occurred from causes quite unknown to the fishermen. The chief provinces where this unfortunate condition has continued to occur are Sado, Oki, Tsushima and Utsuryotô. In all these places, it is said, formerly the cuttlefish were abundant the whole year, being most numerous in summer. In this season the sea is usually quiet as mentioned above, and the fishery can be carried out with ease so that formerly it was very successful, along the whole coast of the islands, so as to bring the total annual catch to a great amount. In recent years, however, this has been greatly changed, the cuttlefish being as a whole very scarce and the season when they are most abundant is not the summer but autumn or even winter, or, as in Sado, at every season they are so scarce that the catch does not pay for the labor. What is then the factor which caused the barrenness of the fishing places and the change of the shoaling mode of the cuttlefish? Not only from the economic point of view but also from the theoretic, we have to clear up this question which forms the final chapter of the present investigation.

Confronted by such a problem as this, one might assume as the causal factors uncontrolled exhausting fishing of the creature under consideration, together with the ecologic changes which may take place in consequence of such an irreplaceable catch as noted. These are, it seems to me, in reality not the principal factors, for the decreased crop in one province is compensated by the increase in others, so that the total catch is not changed. As a good example of the reciprocal change of catch, I refer here to the case between Hokkaidô and the prefecture to which the famous fishing place of Sado belongs, Niigata-ken. In Hokkaidô the annual catch has been increased year after year, while in Niigata-ken the opposite has been the case, showing a decrease roughly proportional to the increase in the former, as seen from the annexed curves (fig. 5).

The further our studies go, the clearer is made the fact that there is no evidence of decrease at all, but rather a great increase, in the total annual catch. As seen in the following table which represents the total annual catch from 1905 to 1917 reported by the Bureau of Agriculture and Commerce:

1905	5,536,889 kan*	1912	16,489,756 kan
1906	6,496,634 "	1913	19,257,698 "
1907	8,333,680 "	1914	20,210,660 "
1908	4,802,423 "	1915	12,095,727 "
1909	5,173,717 "	1916	21,913,438 "
1910	7,726,278 "	1917	17,880,940 "
1911	9,153,038 "		

* 1 kan = ca. 3.8 kilos.

This increase is probably due to the increase of professional population as well as the improvement of fishing methods and statistics. At any rate, however, it is true that there is seen even no tendency towards decrease.

From what is above said, the ecologic changes, if there have really been such changes, can not necessarily be assumed to be caused by fishing without control.

The third factor which comes under consideration is that due to the physical conditions of the sea. Setting aside the daily and seasonal changes, and those, as rarely happens, by volcanic agencies, we see a great factor in the hydrographic

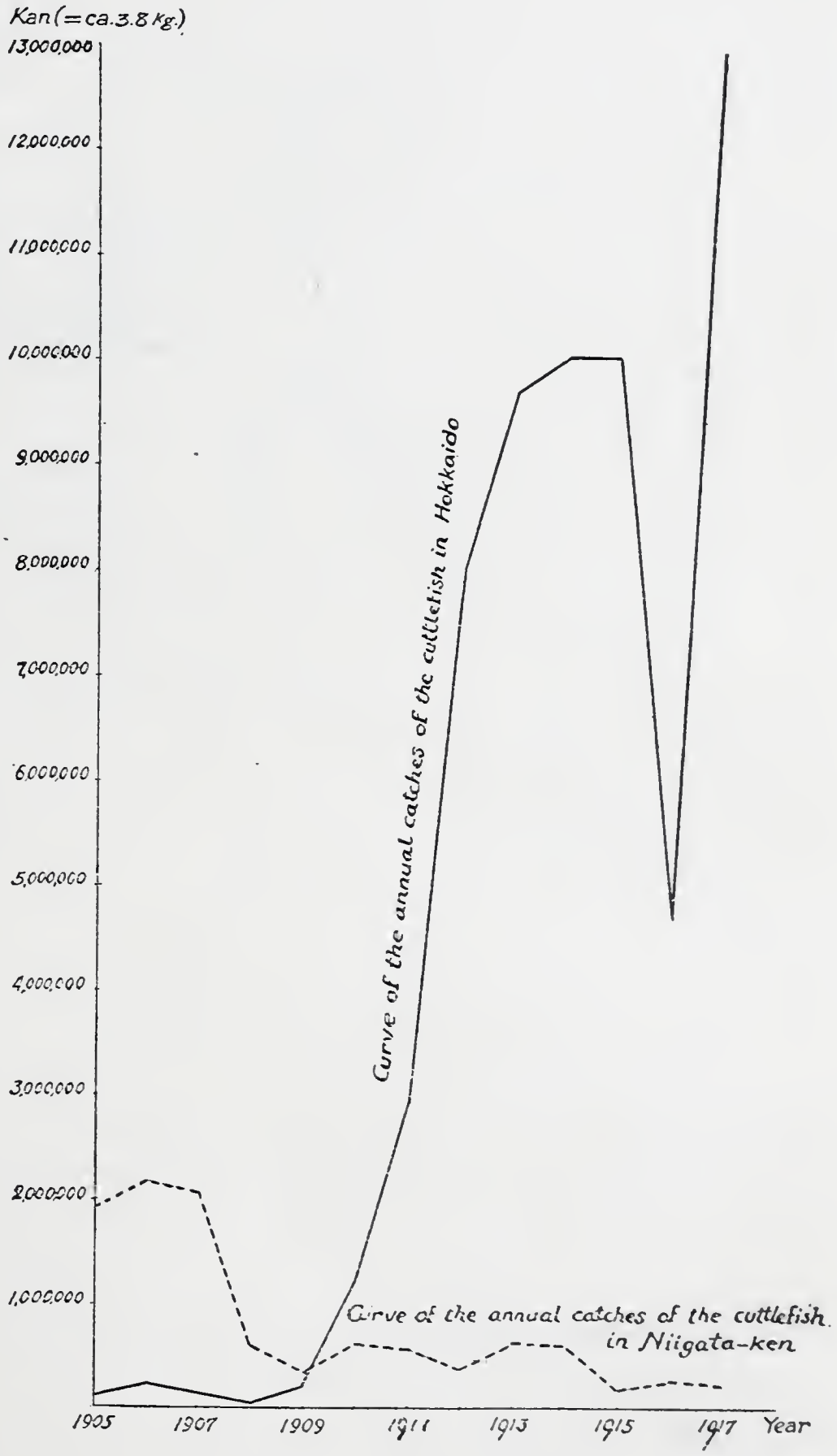

FIG. 5.

Curves of the annual catches of *Nibanzurumê* in Niigata-ken and Hokkaidô, compiled from the Statistical Reports of the Agricultural and Commercial Bureau of Japan.

agencies which regulate and have extraordinary influence upon ecologic life of the animal, consisting in its distribution, migration, and habit of reproduction. It has been considered above, that the seasons, warmer and colder waters, affect the catch; a weak daylight seems also to favor the creature, but the chief natural agency favoring it is a certain temperature that stands between 10° C. and 17° C., as it flourishes best in this temperature of water. As thermal regulators of marine water are first of all to be taken into account the oceanic currents. Let us take a glance at the currents around the Japanese Islands.

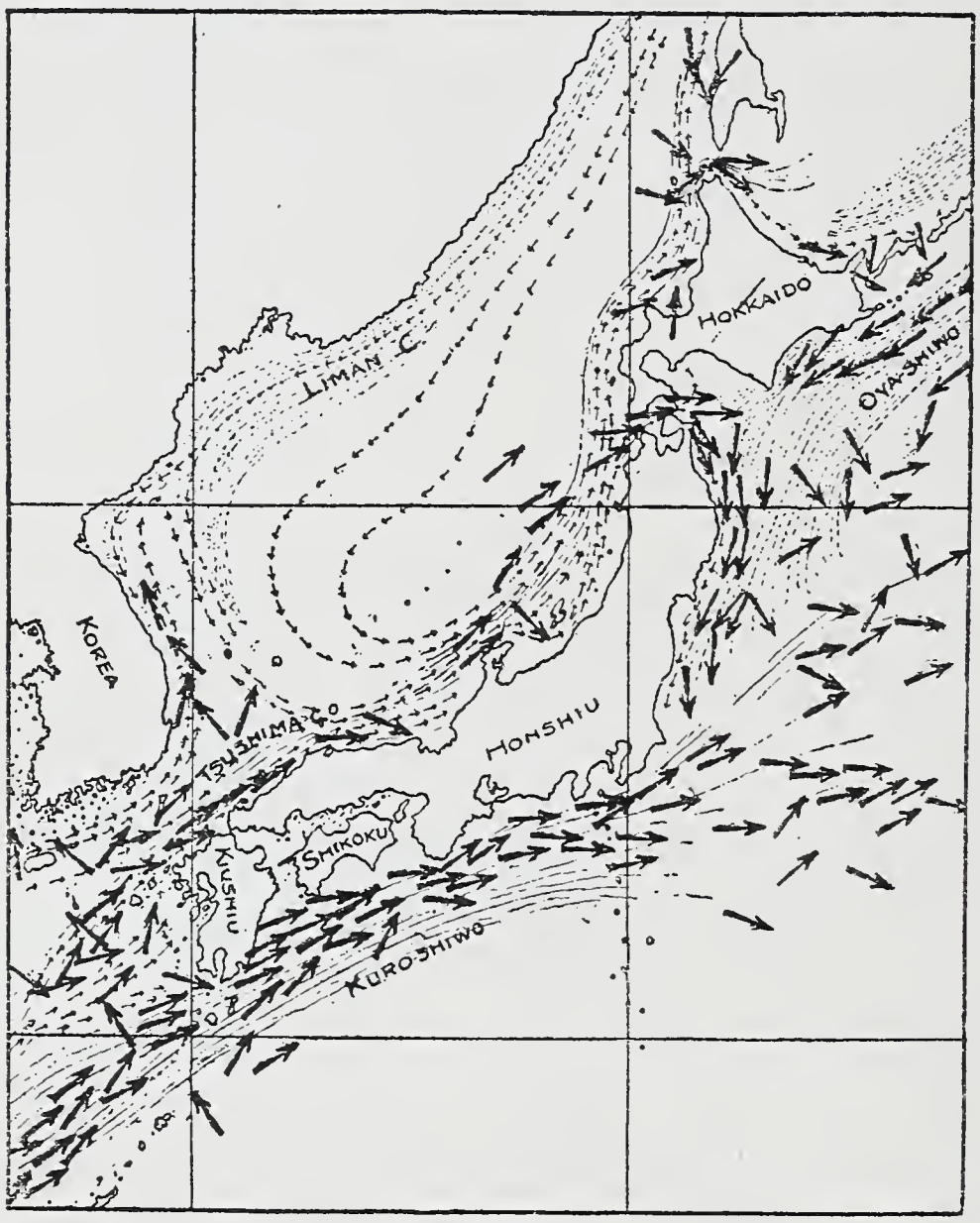

FIG. 6.

Currents in the upper water-layers of the seas around Japan. The smaller arrows are referred to Wada's observations of the drift bottles, and the larger arrows, to the current chart for the North Pacific, Jan.-Dec., published by the Hydrographic Department, Tokyo.

As is very well known, we have on the Pacific side two principal currents of different temperatures (fig. 6). The warmer is the well-known Kuro-shiwo (the Japan Stream) which is peculiar by its high salinity and flows northwards and eastwards along the Loochoo group and the southern coasts of Kiushiu and Shikoku, extending a little more northerly than the middle part of Honshiu. Hereafter, it diverges from Japan so as to form the North Pacific Drift. The colder current is known as Oya-shiwo, which flows west and south along the east coast of the Kurile Group and of Hokkaidô, and reaches the northern part of Honshiu, where it meets with the aforesaid Kuro-shiwo. Both currents vary in strength in different seasons: the Kuro-shiwo is strongest in the warmer season and weakest in the colder, while the reverse is true of the Oya-shiwo; hence the

meeting point of these currents is shifted sometimes up and sometimes down, oscillating along the northeastern coasts of Honshiu.

In the Eastern and Yellow Seas (Tun-hai and Hwang-hai) there is a coastal water of low salinity which, according to Wada's testing of drift bottles (1915, *l. c.*), seems to circulate slowly counterclockwise. It is combined with some branches of the Kuro-shiwo near the Japanese coasts so that the climate of the western coast of Kiushiu is much affected by the high temperature of the Kuro-shiwo. The strength of these branches differs in different seasons: they are very weak in winter as proved by Mr. Marukawa (1918, *l. c.*) but strong in summer, some entering the Japan Sea through the Tsushima Strait.

The Japan Sea is an enclosed basin with four narrow outlets: the Tsushima Strait (Korea Strait), Tsugaru Strait, Soya Strait (La Pérouse Strait) and Mamiya Strait (the Gulf of Tartary). In this sea two currents flow: one which is called the Liman Current, is colder, and flows south along the Amur coast, and the other, which is known under the name of the Tsushima Current, is warmer and runs north along the Japanese coast. Schrenck and Makaroff likewise mention that the Liman Current originates in the Okhotsk Sea, entering the Japan Sea through the Mamiya Strait, and going into the Eastern Sea through the Tsushima Strait along the Korean coast, while the Tsushima Current is a branch of the Kuro-shiwo, which comes into this sea basin through the strait of its name, and goes in part into the Pacific Ocean through the Tsugaru Strait and in part into the Okhotsk Sea through the Soya Strait.

This view is, however, not wholly correct, since the Mamiya Strait is really so shallow as not to let pass such a constant stream as the Liman Current, while the Tsushima Current is distinguished from the Kuro-shiwo, being by no means so high in salinity as the latter. There is, on the other hand, a strong reason to believe that the water of the Japan Sea is not really oceanic but rather coastal, circulating counterclockwise, as is usual in such an enclosed sea in the North Pacific. The said two currents represent, therefore, in most of their parts, nothing but sections of this large circulatory current. Of course, the water is by no means simple or isolated, but compound and connected with those of other seas. For instance, it is combined in the southern corner of the Japan Sea with the warmer water coming through the Tsushima Strait, which water consists, in its turn, of branches of the Kuro-shiwo and the coastal water of the Eastern Sea, so that the said Tsushima Current

is a mixed water from three different sources, of which the relative proportions, of course, vary greatly according to different seasons.

The Tsushima Current flows northward as illustrated by Schrenck and Makaroff, bathing the northwestern coast of Honshiu. At the northeastern part of the Japan Sea it is divided into two courses, one of which passes through the Tsugaru Strait to come out into the Pacific Ocean, and the other goes on its way still northward to bathe the western coast of Hokkaidô. It is a necessary consequence that the Pacific branch strikes against the Oya-shiwo, and then turns itself to the right side as is the rule in the Northern Hemisphere, taking for some distance a southern course parallel with the Oya-shiwo. It is this southern course that has a great significance for our present problem, for along the coast of Iwaté-ken the currents of different temperatures are put in an interesting hydrographic arrangement: the warmer current, *i. e.*, the said branch of the Tsushima Current passes nearer to the coast, as if pressed against the shore by the Oya-shiwo, the colder current. The relative strength of the two has much to do with the crop of some fishes.

The second portion of the Tsushima Current turned northwards gives off a branch passing through the Soya Strait into the Okhotsk Sea, while the main current extends to the west coast of South Sakhalin to turn round, as it is highly probable, to contribute to the formation of the said Liman Current.

Having given a rough sketch of the oceanic currents around the Japanese group of islands, we turn to inspect how these affect the distribution of the cuttlefish. Fig. 1 is a map intended to show the density of population of the animal. As the representation is simply based upon the statistical annual amount of catch, it is clear that the density shown with dots on the map does not exactly express the actual occurrence of the animal, but is represented at a measure lower than actual in some localities, for instance, as in the coasts of Yamagata-ken and Akita-ken, owing to comparatively small crop brought about by reason of several circumstances, under which the cuttlefish-fishing can not profitably be carried out. Putting aside such an apparent rupture of the zone of population, the density describes, first of all, a zonal curve with the densest part in coincidence with the extent of the Tsushima Current which contains in a proper depth of water strata of 10°–17° C. (fig. 7) favorable to the life of cuttlefish, as seen along the northern coasts of Honshiu and also on the coast of Shimané-ken, where a great catch of the cuttlefish was made throughout August of last year. The accompanying hydrographic section

shows the results of the observations carried out, just at the beginning of the catch, by an official experimental boat along a line extending 100 miles off the coast. A warmer water of the Tsushima Current, the lowermost stratum of which is nearly of the temperature best suited for the cuttlefish to live in, is found, as seen in the section, close to the coast, being very probably pressed from the oceanic side by a big body of colder bottom water of the Japan Sea.

As mentioned before, the warmer water of the Tsushima Current comes out into the Pacific side of the northern part of Honshiu, passing through the Tsugaru Strait and bathes the coast of Iwaté-ken. In this place, when the current is strong as in summer, it extends over the Oya-shiwo of the offing, and when the latter is enlarged as in winter, the former is compressed into a narrow zone towards the shore. The seasonal population of the cuttlefish in this prefecture is much connected with this relative strength of these two currents.

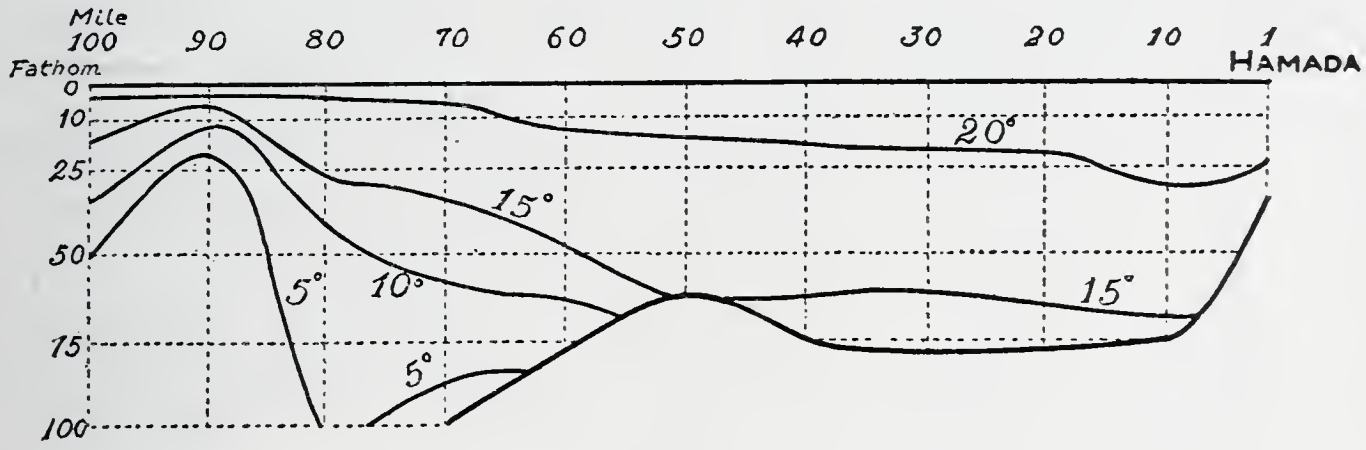

FIG. 7.
Hydrographic section off Shimané-ken, showing vertical distribution of temperature on July 14–15, 1919 (from a hydrographic observation made by the Fishing Experimental Station of Shimané-ken).

This is shown by an unusually good catch taking place 5–10 miles off the coast in the latter part of October of last year, when an official boat of the local government was carrying out, along a line extending 80 miles off the coast, observations the results of which are represented in the section shown in fig. 8. There appears close to the shore in the section a zone of warmer water of 12°–16° C. which represents nothing but the said branch of the Tsushima Current inhabited by the cuttlefish. In the offing there is a large water body illustrated by isothermal lines of 4° C. and 8° C.; this is probably the Oya-shiwo, which is of a temperature lower than that best for the creature under consideration. This current which was just enlarging at that time, as occurs usually in later autumn, seems to have strongly pressed the warmer current against the shore into a narrow, but deep vertical zone of roughly 10 to 20 miles in breadth, where the said good catch was made. Under such a hydrographic condition it is probable

that the cuttlefish, that were found scattered over the horizontal extension of the warmer water when this was expanded, are carried up with the water in which they lived, so as, so to speak, to be crowded in the narrow zone just mentioned and can easily practice their daily vertical migration, having temperature suited for themselves at every stratum. As it is in coincidence as regards the season, the large catch above referred to can certainly be employed as an illustration of this occurrence.

Let us now examine the ecologic relation of the cuttlefish to the two other currents, *i. e.*, the Oya-shiwo and Kuro-shiwo. With regard to the first

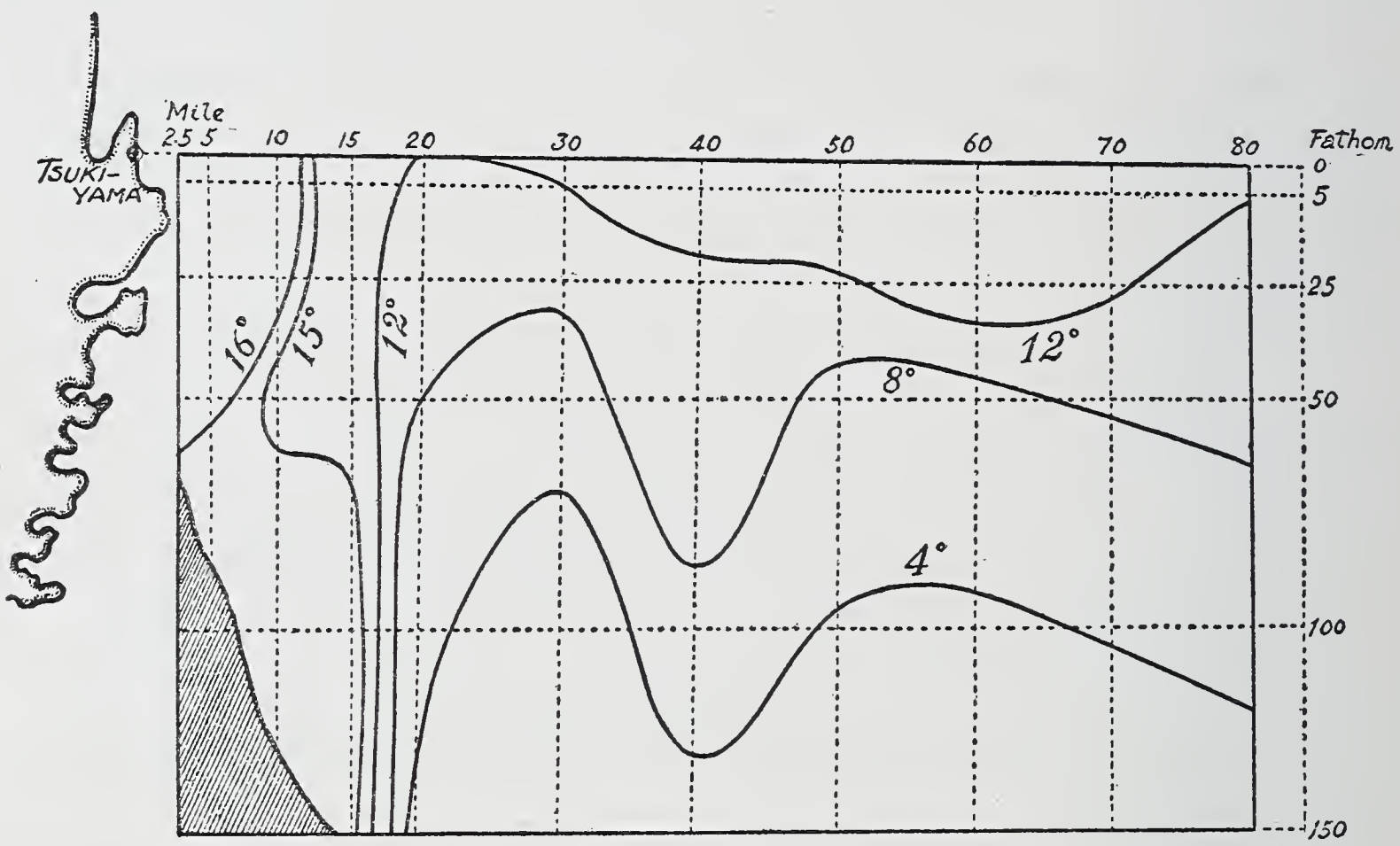

FIG. 8.
Hydrographic section off Iwaté-ken, showing vertical distribution of water temperature on Nov. 1, 1919 (from a hydrographic observation made by the Fishing Experimental Station of Iwaté-ken).

current, I have already alluded above to its relation to the distribution of the animal. In a comparison of the map of currents (fig. 6) with that of the population of the creature (fig. 1), one would not hesitate to recognize that this current is by no means favorable for the cuttlefish, owing, as mentioned above, chiefly to its low temperature. The unfitness of the colder current in this respect is most obvious at the coast of Fushima-ken, and also at the eastern coast of Hokkaidô (Tokachi): both the places stand alike at the front of the lands, against which the current strikes, flowing straight down from a region east of Kamchatka.

Concerning the latter locality, a zone of relatively warmer water in which

the cuttlefish may live is recognized at Nemuro along shore, as inferable from the taking of a good catch of the cuttlefish in this water zone, together with several fishes proper to warmer waters, such as bonitoes, which sometimes make a large part of the product of the fishing villages about the town of Nemuro. It can not at present, however, be told with certainty whether this body of water is a branch current from the Pacific or the Okhotsk division of the warmer water crossing down the Nemuro Canal. In the eastern section of the coast of Kitami the population zone of the cuttlefish is also broken off, owing either to small fishermen population or to the presence of the Okhotsk water of very low temperature, as illustrated by Schrenck.

Occurrence of the cuttlefish is also very rare along the coasts of Ishikari

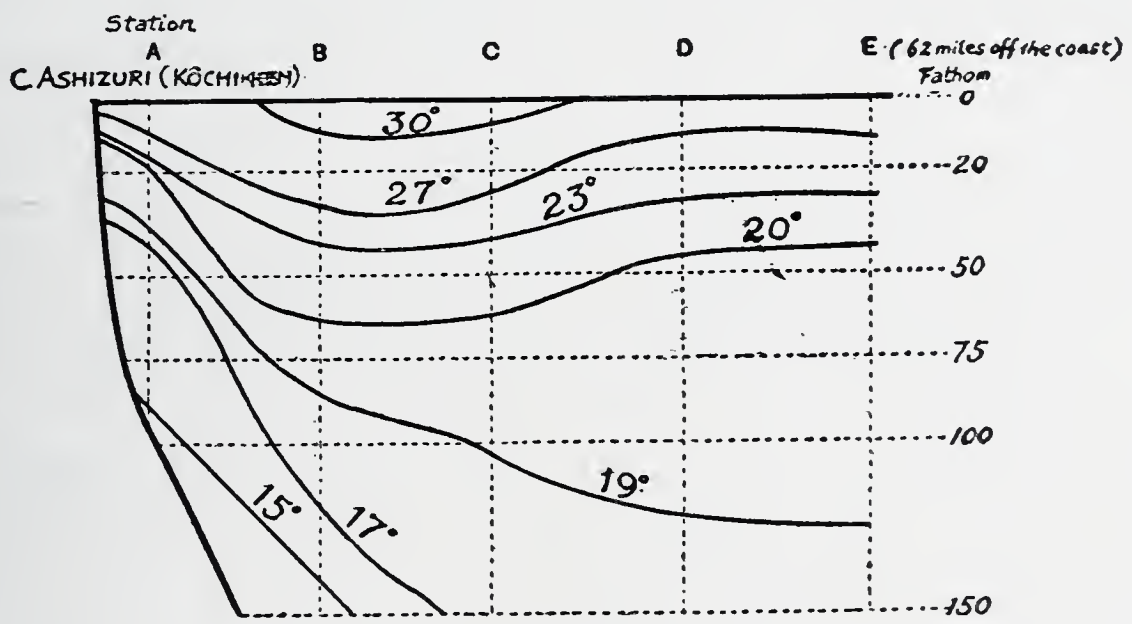

FIG. 9.
Hydrographic section off Kochi-ken, showing vertical distribution of water temperature on July 25–31, 1917 (from a hydrographic observation made by the Fishing Experimental Station of Kochi-ken).

and Teshiwo, both lying at the western side of Hokkaidô; this is, however, not from the influence of the colder water above referred to, but because the littoral zone is too shallow for the cuttlefish and also because fishermen are engaging in much more profitable pursuits, such as salmon fishing.

The second current, the Kuro-shiwo, is to be shown also as unfavorable for the animal to live in (see again figs. 1 & 6), owing in the main doubtless to its high temperature. The southwestern coast of Kiushu, as well as the southern coasts of Shikoku (comprising Kôchi-ken and Tokushima-ken), Wakayama-ken, and some provinces of Tôkaidô like Miyé-ken and Kanagawa-ken, all of which the current bathes, afford best illustration of the assertion.

It is, however, sure that cuttlefish are caught in a certain profitable amount wherever water strata of 10°–17° C. are found on the sea bottom close

to the coast. An actual case is represented by the government of Kôchi-ken, where it is recorded that good catches of the cuttlefish have often been made (fig. 9). The water strata in question lie, as seen in the section, in such a position as just mentioned and parallel with the contour line of the coast. It is not unreasonable to assume that this stratification of water is brought about by water strata of higher temperature, *i. e.*, Kuro-shiwo, pushed towards the shore of rapid inclination, as quite obvious in the section just referred to.

From what has been stated, we do not hesitate to assume that the cuttlefish occur in an ecologic relation which is brought about first of all by a water temperature of 10°–17° C., and that the reason a large body of the Tsushima Current is most profitable for cuttlefish fishing is due principally to its having this favorable temperature in moderate depth, *i. e.*, 50–100 fathoms.

SUMMARY

The facts stated above may be summarized as follows:

(1) The cuttlefish inhabits, around Japan, on the whole the deeper parts of the coastal water (*s. l.*) of a definite temperature, so that in the Japan Sea it is thickly and widely distributed, its thickest distribution roughly coinciding with the extension of the Tsushima Current. In the Pacific Ocean, on the other hand, the distribution is usually limited to the deeper part of the oceanic margin, being brought about by the pressure of the oceanic currents like the Oya-shiwo and Kuro-shiwo.

(2) The animal lives in the coastal waters chiefly because they have a temperature 10°–17° C. in proper depth, 50–100 fathoms, which affords the ecologic conditions most suited for the creature, but in exceptional cases the animal may be adapted to colder as well as to warmer water, as it is the case in northern and southern seas.

(3) The horizontal migration of the cuttlefish is not so wide as from Kiushiu to Hokkaidô, but limited to a relatively small sea area. Its vertical migration takes place every day, extending from 50–100 fathoms in daytime to 0–20 fathoms at the twilight of sunset and sunrise. During the daily vertical migration it seems to be not greatly affected by the change of water temperature met with in its travel through different water strata.

(4) The cuttlefish becomes mature in a year. Young individuals live on floating micro-organisms, but when grown their food consists chiefly of living

fishes. The plankton-feeding young are found most frequently in spring, with their mantle measuring below 85 mm. in length.

(5) In warmer seas the principal pairing season continues from summer to winter, but in colder seas it occurs only in early winter.

(6) The male sticks the spermatophores on the buccal membrane of the female, the membrane having about thirty seminal receptacles arranged in a circle around the mouth.

(7) In warmer seas the *principal* spawning season occurs as a rule in autumn, even though the spawning may take place nearly all the year round.

(8) It is highly probable that the eggs are deposited on the sea bottom and embedded in a gelatinous mass.

(9) The artificially fertilized eggs which develop to a certain extent are much heavier than sea water and repose on the bottom of the basin. Their cleavage is partial, somewhat resembling that in *Loligo*.

(10) The habit of the cuttlefish is to a large measure regulated by the hydrographic condition of the sea, changes of which stand in relation to the large or poor catch of the animal.

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ELM GROVE

Residence of William Wagner, where he began public lectures. The building was torn down in 1886

WAGNER FREE INSTITUTE OF SCIENCE

Montgomery Avenue and Seventeenth Street

TRANSACTIONS OF THE WAGNER FREE INSTITUTE OF SCIENCE

OF

PHILADELPHIA

VOL. X

OCTOBER, 1923

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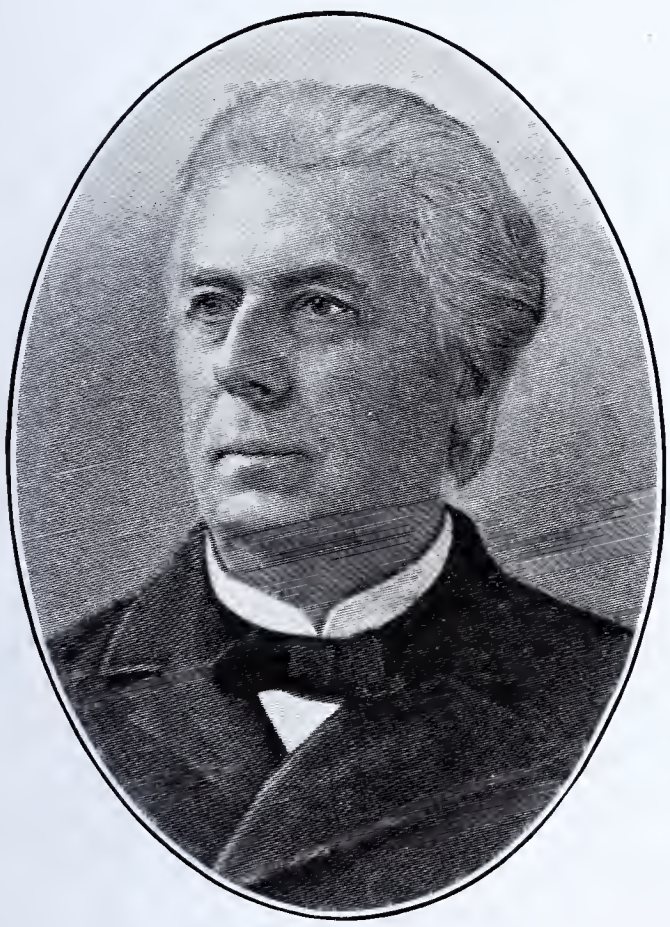
CONTENTS

William Wagner—1796–1885

Louisa Binney Wagner—1814–1898

Founders of the Institute



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Trustee, 1884–1899

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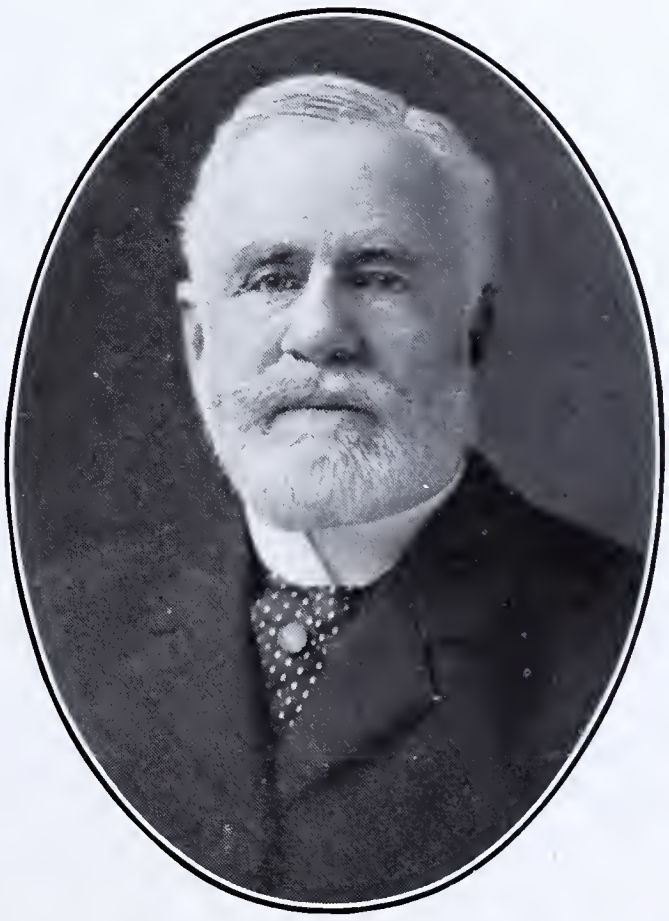
First President of Faculty
1885–1891



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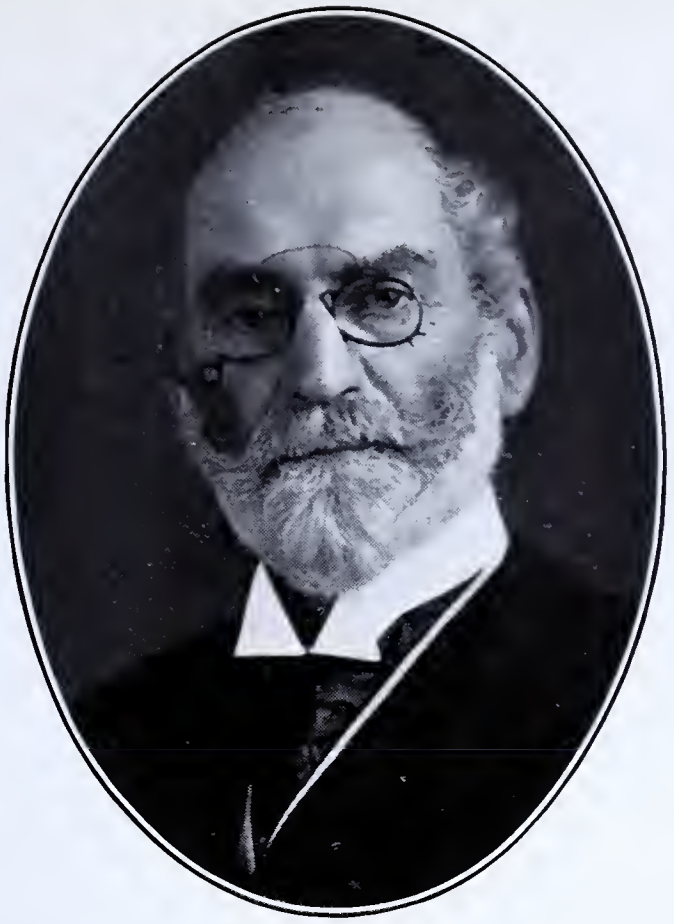

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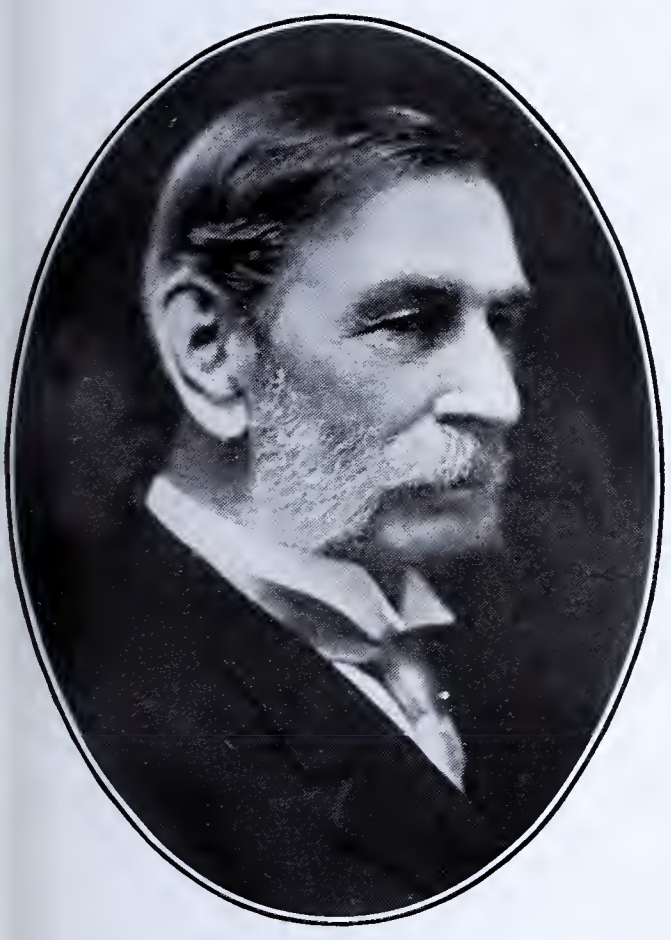

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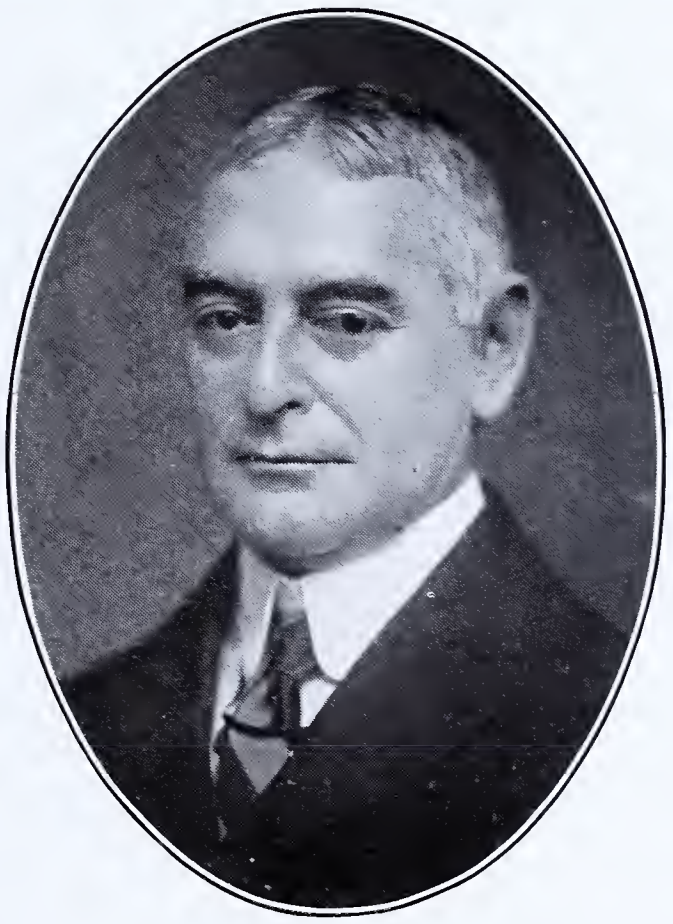

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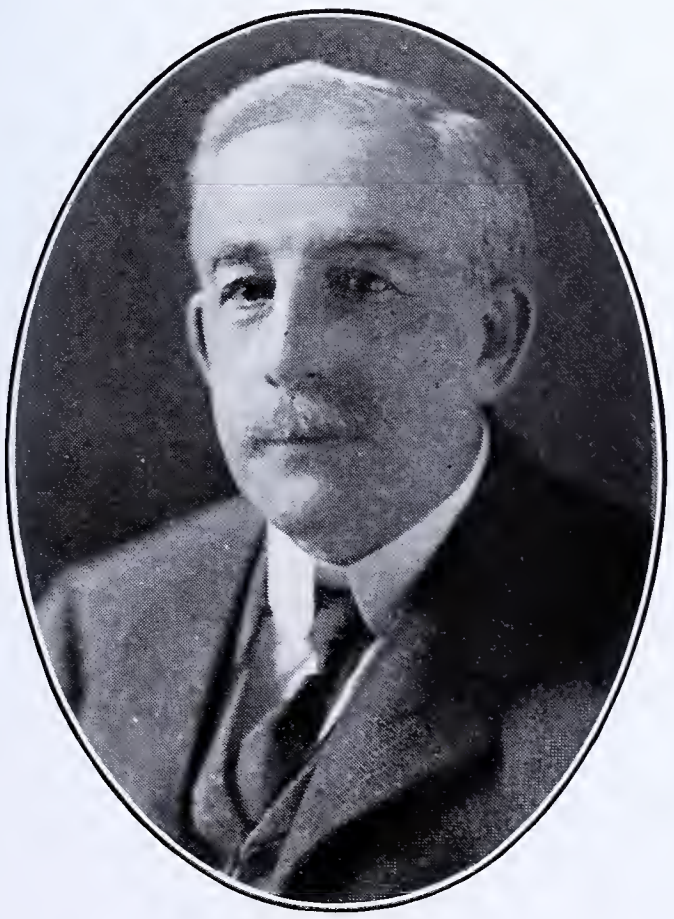

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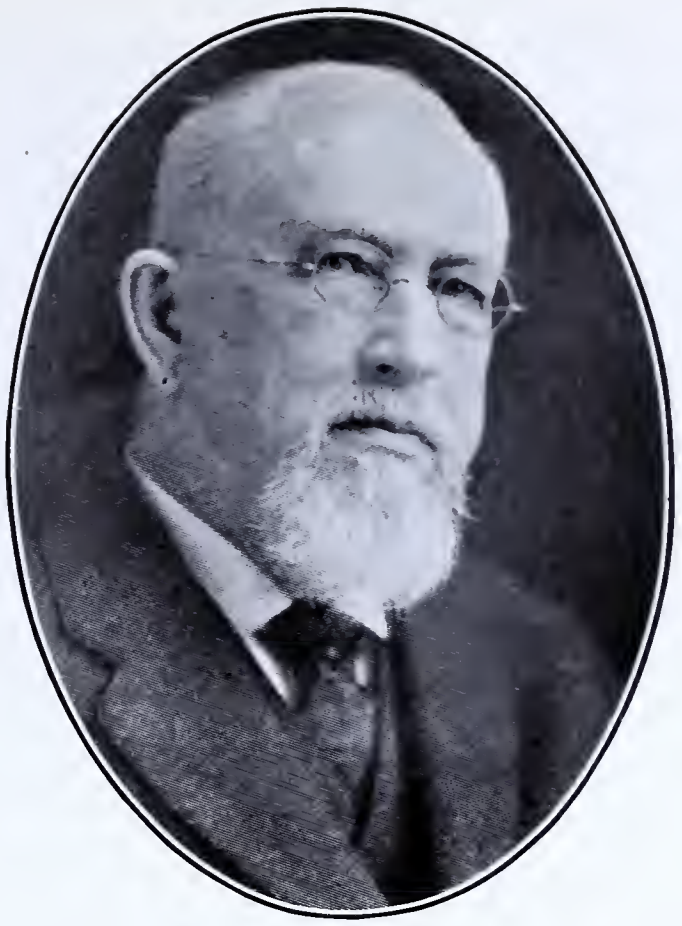

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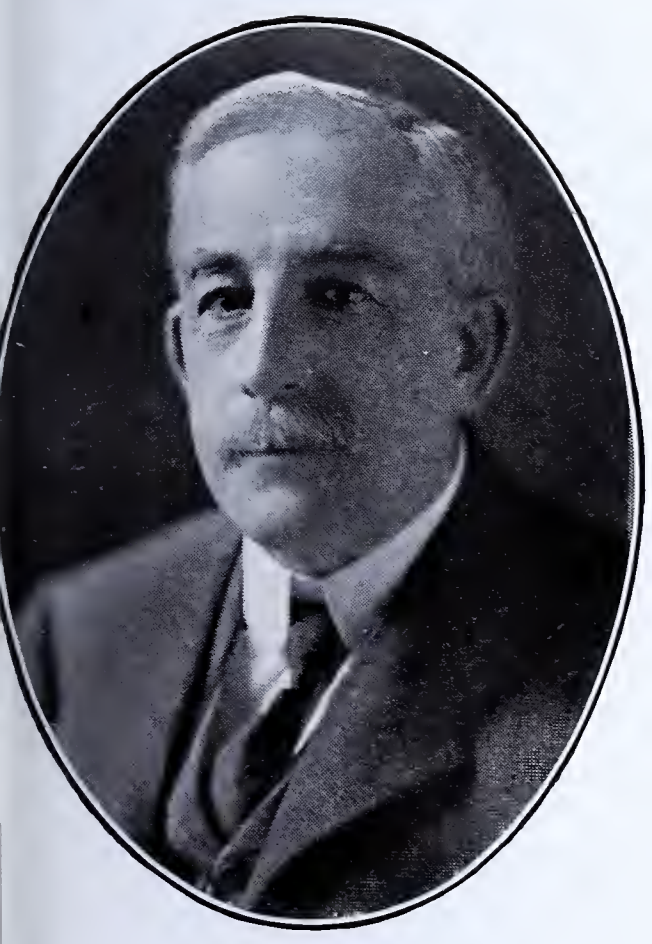

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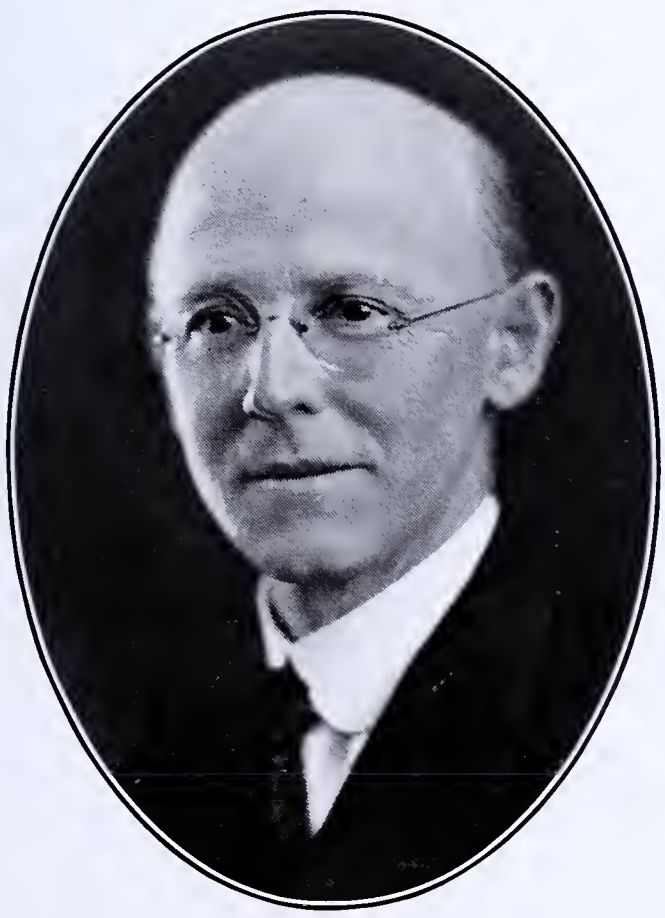

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THE WAGNER FREE INSTITUTE OF SCIENCE OF PHILADELPHIA

PREPARED BY HENRY LEFFMANN, WITH THE COLLABORATION OF JOSEPH WILLCOX AND SYDNEY T. SKIDMORE

[Reprinted with some modifications from Founders' Week Memorial Volume, Philadelphia, 1908]

THE Wagner Free Institute of Science owes its establishment to the liberality and public spirit of William Wagner and his wife, Louisa Binney Wagner. In his early life Professor Wagner made extensive voyages in the service of Stephen Girard, and had opportunities to visit scientific institutions and make the acquaintance of scientific workers. He soon developed a strong interest in the natural sciences, especially geology and mineralogy, and devoted a large part of his life to studying these topics and collecting material to illustrate the teaching of them.

As early as 1847 he began to give lectures at his private residence, located in Penn Township, in the northwest suburbs of the city. The estate, which Professor Wagner had recently purchased, was known as Elm Grove. It faced on Turner's Lane. The rural character of the district is indicated by one of the early notices of lectures, which states that the entrance is at the "corner of Turner's Lane and Stump Lane." This locality was years ago absorbed by the city, and is now a built-up area.

The Institute began its corporate life in 1855, when an Act of the Legislature of Pennsylvania conferred upon Professor Wagner, in association with William H. Allen, James Bryan, Robert E. Peterson, and George M. Keim, powers as trustees in the usual form. Later, a supplementary act was passed, but the scientific features of the Institute's history were not affected by these amendments. The collections and library in possession of the corporation had increased so as to render it necessary to secure for them more room than the private house afforded, and moreover the distance from the city, with the then imperfect methods of transportation, greatly restricted the usefulness of the work. Hence the founder sought, with his customary zeal and energy, for a convenient building and succeeded in securing the hall at Thirteenth and Spring Garden Streets. This had been erected by the District of Spring Garden for its business offices, and in accordance with the Act of Consolidation of 1854 had become the property of the city of Philadelphia. The first course of lectures was begun in the spring of 1855. An active schedule was inaugurated at

that time, two lectures being given each evening, the periods being from 7 to 8 and from 8 to 9. Professor Wagner was a member of the faculty, lecturing twice a week. The charter of the Institute is of a comprehensive character, and in the course of its existence it has presented in the form of lectures and class-work a great range of scientific subjects.

A notice in possession of the Institute gives the following list of lectures, being probably the first faculty:

Professor Wagner, Geology, Mineralogy, and Mining.
Professor Stephens, Natural Philosophy.
Professor Houston, Human Anatomy.
Professor Child, Physiology.
Professor Foster, Zoology.
Professor Cummings, Architecture.
Professor Dickinson, Entomology.
Professor Walker, Chemical Agriculture.
Professor Bryan, Comparative Anatomy.

No professor of chemistry proper was named, although an hour was assigned for the subject.

In March, 1859, the city authorities were obliged to withdraw the permission for the use of the hall on account of the need of it for municipal uses, and it became necessary for another location to be secured. After making inquiry as to available sites, Professor Wagner decided that he would erect a suitable building within the bounds of his own property, at the corner of Seventeenth Street and Montgomery Avenue. Considerable opposition was made to this by many persons on account of the distance from the city, but the founder of the Institute felt that he was not building for a season, but for a future, and his practical mind saw that not many years would elapse before the neighborhood would be an integral part of the great and growing city. The immediate locality offering a good supply of clay, Professor Wagner arranged to make the necessary bricks, and succeeded so well that on June 2, 1860, the cornerstone of the new edifice was laid, with proper ceremonies. Among the distinguished speakers of the occasion was William D. Kelly, then a judge in the city court, afterward well known in Congress for his advocacy of a protective tariff, especially on iron products, which gained for him the nickname of "Pig Iron Kelly."

The Civil War interfered with the progress of the building, but on May 11,

1865, the completed edifice was formally transferred to the trustees, and on the following Monday, May 15th, the first lecture was delivered at 5 P. M.

At this period the building was still in the suburbs, and was reached by a long boardwalk running in from Columbia Avenue. The residence was still undisturbed on the ground to the northward of the building. The surroundings were pleasant and, notwithstanding the distance from the built-up city, many persons attended the lectures. Before long the system had been adopted of having one lecture each evening, and a different subject each day of the week. The course was arranged in two periods, divided by a considerable interval at the winter holiday season. The names of many persons who have since become distinguished in science are to be found upon the lists of lecturers at this early period. Among these many be mentioned Dr. Charles K. Mills, Dr. DeForest Willard, and the late Dr. William H. Wahl.

Professor Wagner was, for a long while, an active member of the faculty, lecturing on geology and mineralogy. In later years, desiring release from this arduous duty, he relinquished the work to others, but he was almost always present as a listener at the lectures.

Two special developments in Institute work for which the opportunities were seemingly not ripe may receive brief notice.

As noted above, the charter of the Institution is a comprehensive one, giving all the powers usually accorded to American educational institutions. The founder intended to combine an institute for collegiate training with the advantages of a public library and museum, and especially public lectures on scientific subjects in form adapted to a general audience. This last was, indeed, one of his most cherished purposes. In 1865, he decided to start a regular polytechnic course. He accordingly organized a faculty and issued an announcement. A comparatively small fee was fixed for tuition. The plan did not succeed, and it was entirely abandoned soon after.

The following is a list of the faculty and departments intended for this course:

William Wagner, Professor of Geology, Mineralogy, and Mining.

Andrew E. Rogerson, Professor of Civil and Military Engineering, Mathematics, Surveying, Levelling, Navigation, Mechanical Drawing, and Astronomy.

Charles A. Leish, Professor of Anatomy and Physiology.

Charles S. Gauntt, Professor of Chemistry and Natural Philosophy.

N. K. Richardson, Professor of Elocution.

The collegiate year began September 18th and closed May 18th.

At a later period Professor Wagner, at the suggestion of Professors Wahl and Grimshaw, organized a "Mechanics' Institute." This consisted of a meeting once a month, in which notices of new inventions and discoveries in the arts and sciences were announced, and new forms of machinery and other apparatus exhibited. The meetings were, of course, public, and held in the lecture hall. For this, also, the conditions were not favorable, and the work was soon discontinued.

The lecture course of the Institute continued in the regular way for many years. In 1885, a change took place in consequence of the death of the founder, which occurred on January 17, 1885, two days after entering upon the ninetieth year of his life. Under the charter, the powers of the trusteeship had been wholly in his hands during his life. Now, by his death, the entire board became entrusted with the duties of control. These trustees were Samuel Wagner, Joseph Willcox, Richard Brodhead Westbrook, Sydney T. Skidmore, J. Vaughan Merrick, and Samuel H. Cramp.

On May 30, 1864, Professor Wagner and his wife, Louisa Binney Wagner, had jointly executed a deed of trust conveying certain properties to the corporation for the uses of the Institute. In this deed, as well as in the charter, the availability of the opportunities of the Institute is based upon the broadest lines; the benefits of the teaching and the opportunities of holding positions in the Institute are not to be influenced by race, religion, nationality or sex.

It was to the administration of this noble and comprehensive scheme of education that the trustees above named succeeded in the early part of 1885. They immediately began to arrange for enlarging and extending the work of the Institute, and to this end selected a faculty. They invited Dr. Joseph Leidy to assume the presidency of this—an invitation that he accepted. The other members were Henry Leffmann (Chemistry), Angelo Heilprin (Geology and Paleontology), and Benjamin Sharp (Biology). The building was thoroughly reconstructed and improved both within and without. The museum was provided with new cases, many new specimens were obtained, and the lecture hall was refitted. Thus equipped, the Institute has been carrying on the lecture work since that date without intermission.

As soon as possible a department of research and publication was established. The first work undertaken was an expedition to the west coast of Florida by Mr. Joseph Willcox, who was familiar with the territory to be explored, and Prof. Angelo Heilprin. Much valuable information as to the

paleontology and geology of that region was obtained, and the data were presented in the form of the first volume of "Transactions," a royal octavo containing 134 pages of text and 18 plates, entitled "Explorations on the West Coast of Florida and in the Okeechobee Wilderness," edited by Angelo Heilprin. Through this and succeeding publications, the Institute secured numerous accessions to its library by exchange.

When the educational system, termed "Extension of University Teaching," was introduced into Philadelphia, the Wagner Free Institute was one of the most active friends of the movement, and several courses of instruction were organized under its auspices. Subsequently, the special "Extension Courses" passed into the control of the society organized for that purpose. The Institute continues to afford the free use of its lecture hall and library for such courses as are assigned to it. As a result, however, of the extension work, the regular lecture courses of the Institute adopted, and have continued, a system of class-work of either written or oral recitations. From the records made by the students in this line, certificates are issued. The usual arrangement is for the complete course in each department to extend over four years. At the close of a full course those students who satisfactorily performed the work are given full-course certificates.

Several years after this, overtures were made by the Institute to the Board of Public Education for the establishment of a Free Public Library at the Wagner Institute with funds that had been appropriated to the Board of Education for that purpose. A successful working arrangement was adopted which continued with the Free Library under the direct management of the actuary of the Institute, until the Board of Trustees of the Free Public Library was organized and the control of the library was transferred to it by the Board of Education. This library, as the parent branch of the Free Library system of Philadelphia, has been continued to the present time.

Since 1901, the circulating department of this library has occupied a commodious building erected especially for its use, while the reference department has been combined with the reference library of the Wagner Institute and established in quarters in the original building, convenient of access.

With the opportunities that his journeys in connection with his business trips afforded him in early life, Professor Wagner secured many valuable specimens of fossils and minerals, visiting many of the localities in which these were found. Subsequently, he continued this collecting with great zeal, and as a

result the Institute collections in these two departments are valuable and prominent features of its museum.

By purchase and exchange the Institute has acquired, of late years, additional museum collections, embracing illustrative material in all departments of natural history. These collections, housed in a commodious and well-lighted hall, are accessible freely in the afternoons of Wednesday and Saturday throughout the year, except legal holidays.

The large collection of books in the library of the Institute, embracing all departments of natural science and considerable material on general literature and general science, is accessible, without charge, during convenient hours each business day.

It will be seen by this sketch that the personal labors and self-sacrifice of Prof. William Wagner have resulted in the founding of an Institution which has realized all the hopes of its founder, and which may be expected to continue to be an effective agent in the spread of true knowledge, so important in our modern civilization. In considering this matter the assistance rendered by Mrs. Louisa Binney Wagner must not be overlooked. She seconded her husband's efforts in every way she could, and in joining with him in the granting of the deed of trust, placed all the beneficiaries of the Institute under obligations to revere her memory with that of the founder himself.

It was the especial wish of the founder that admission to the popular lectures, museum, and library should be free to all. This wish has been carried out and access to these departments of the Institute is obtained without even the formality of tickets; nor is any charge made to those who enter the classes held in connection with the public lectures, either for tuition or for certificates.

By the will of Richard Brodhead Westbrook, for many years a trustee of the Institute, an endowment for special lecture courses was established. This was subject to the life interest of his widow, Henrietta Payne Westbrook, who by her will increased the principal sum of the endowment. The income thereof was directed to be used to support lecture courses on topics of interest and importance to the public. The administration of the fund is in charge of the Institute, and under it courses have been given each year by distinguished scientists.

A friend of the Institute has established a fund, the income of which is to be used for research in chemistry and allied sciences, and for purchase of apparatus for the lecture courses and special investigations.

Information as to the lectures and publications under these special funds will be found in the annual announcements of the Institute.

STUDIES IN POST-FIXATION DEVELOPMENT

HENRY LEFFMANN
[Contribution from the Research Laboratory of the Wagner Free Institute of Science]

LIGHT has probably an effect on all substances, but only in a few cases is the action so prompt and evident as to attract notice, or to be of practical value. During the 70's of the last century a case of chemicals was presented to the Central High School of Philadelphia by a manufacturing firm. The case, which was provided with glazed doors, was placed in the hall of the school, where at certain times the sunlight struck it. At my suggestion William Beam, a pupil in the school, examined the chemicals and found that many of them showed marked difference in color between the front and back portions, thus showing the effects of long action of light. So far as now known silver compounds show the greatest susceptibility to light, and for this reason have been the main materials of photography. The chlorid, bromid and iodid are the most sensitive. These are commonly grouped under the term "silver halids." Silver chlorid responds promptly to the action of light, becoming discolored, but the other halids show no visible change unless the exposure is prolonged. A brief exposure, even a very small fraction of a second, is sufficient to modify the bromid or iodid in such a manner that by certain chemicals a deposit of metallic silver can be obtained on all parts which have been "light-struck." The condition produced is termed the "latent image," and the procedure by which metallic silver is deposited is known as "development." Much investigation and theorizing have been given to determining the nature of the latent image. Two views have been especially advocated. It has been supposed that a limited number of molecules of silver halid are decomposed, the halogen escaping and the metal remaining in a colloidal state. The other theory is that no complete decomposition of any molecule occurs, but a partial loss of halogen, so that a sub-halid is formed. It has been proved that the action of light on these silver salts is attended by evolution of the halogen, but, of course, such effect would be explicable under either theory.

In actual practice the silver compound is mixed with some substance that can be spread in a thin layer on the support, and in the majority of cases this is gelatin. The fact that silver compounds blacken when exposed to the

air, especially when in contact with organic matter, has been known for several centuries. Glauber, in the latter half of the 17th century, refers to such action, but for a long while it was supposed to be due to air or to heat. In 1727, Heinrich Schulze, by a simple experiment, proved that the discoloration of silver salts was due to light alone, but he did not utilize this information for any practical purpose. In 1839, Daguerre's method was made known by a report by Arago to the French Academy of Sciences, and the art of making pictures by light took a practical position. Many improvements followed, one of the most important being the invention of the "dry plate" in which an Englishman, Dr. Maddox, had a predominant share. This permits the keeping of the plate for some time after its manufacture and also deferring development. Photography at a distance from the studio thus became very easy. A large part of the present vogue of the camera is dependent on this property of the dry plate.

A dry plate being given a normal exposure gives no evidence of any change; the yellowish emulsion of the silver halid and gelatin is apparently not affected, but immersion in a developer soon causes the appearance of an image, which may be carried to any desired degree of intensity. This deposit is metallic silver, due to the decomposition of the light-struck halid by the developer, but that portion of the silver compound which has not been under influence of light remains undecomposed, and if the plate is now washed and exposed to light, this unchanged portion will slowly darken and the picture will be confused. To prevent this, the plate is fixed by immersion in some solution that dissolves the silver halids, but not metallic silver. The best fixing agent is one of the soluble cyanids, particularly potassium cyanid, but they are poisonous and their use is rare. Sodium thiosulfate, known to the photographer as "hypo," on account of the fact that it was erroneously called "hyposulfite," is the agent commonly used.

An ordinary dry-plate immersed in a 20% solution of this fixing agent will soon be apparently completely cleared of the silver salt, and on washing and drying will be perfectly transparent and seem to be nothing but a film of gelatin spread on the glass. This result will be obtained whether the plate has or has not been exposed to the light, provided, of course, that no developer has been used. It would seem, therefore, that after treatment in the fixing bath there would be nothing of the silver salt left and in the case of an exposed plate all trace of the latent image would have been destroyed. Experiment,

however, shows that, with many forms of plates, the latent image is left in vigor after apparently complete fixing. This curious fact has been known for some time, but has been but little studied, because it has seemingly no practical value. It is more tedious than the ordinary method and is liable to yield imperfect results. Still it seems worth while to investigate some of the features of it, and the present paper offers the results of numerous experiments in this line.

It is obvious that development of the image after fixing cannot be accomplished by the ordinary methods, since these depend upon the conversion of the silver salt into metallic silver. The fixing has removed all but minute traces of the silver compound. Yet experiment shows that a distinct impression remains in the film embodying the most minute detail of the latent image. To develop this it is necessary to produce a slow and regular precipitation of some metal upon the surface of the gelatin, under which condition a selective action occurs and a picture is obtained. The metal chosen must be easily reducible, and we are, therefore, practically limited to the so-called "noble" metals, among which mercury and silver are the most suitable.

As is usually the case when we investigate the history of a discovery or invention we find foreshadowings that render very difficult any decision as to the real discoverer or initiator. A somewhat extensive investigation of the general procedure of post-fixation development was made in 1911 by A. & L. Lumière and Seyewetz, who set forth the main features of their results in a paper before the French Society of Photography, published in the Bulletin thereof (*Bull. Soc. Fran. d. Phot.* [3], 1911, **2,** 264, 373). Earlier allusions are to be found in Eder (*Ausführl. Handb. d. Phot.*, 2nd ed., **11,** 45). From this latter account it is learned that Jung in 1858 succeeded in thus developing a collodion plate, and in 1894 Kogelmann obtained results with silver nitrate, using ferrous sulfate or pyrogallol as reducer.

The French investigators term the procedure "physical" development, but give no statement justifying the use of this term. It is true that in the present state of the physical sciences the distinctions between "physical" and "chemical" changes are much less accentuated than formerly, and the extensive development of physical chemistry has practically absorbed chemistry into physics, yet the phenomena of development after fixing seem to be as much chemical as the ordinary procedures in development and fixing, but the matter is not important and need not be further discussed.

The studies about to be set forth start from the communication by LL. & S. noted above. Their paper shows some of the objectionable features unfortunately too common in French photographic literature, a lack of frankness and explicitness. Their studies were limited to the use of mercury and silver as detectors of the latent image. Gold and platinum would doubtless give results, but their cost and easy reducibility make trials of them of little importance. The following is an outline of the procedure that LL. & S. give with mercury solutions.

The plate is given a good exposure and at once immersed, in a safe light, of course, in a 2% solution of sodium thiosulfate. When the silver halid is apparently all dissolved, the plate is well washed and immersed in the developing solution. This solution has to be prepared as needed by mixing two solutions that are separately fairly permanent.

A.	Mercuric bromid	0.9 grm.
	Sodium sulfite (dry)	18.0 "
	Water	100.0 c.c.
B.	Metol	2.0 grm.
	Sodium sulfite (dry)	2.0 "
	Water	100.0 c.c.

For use, 1 volume of B is added to 5 volumes of A. The plate is immersed and allowed to remain until the image is sufficiently developed. The development may be carried out in daylight. No explanation is given as to why mercuric bromid is chosen in preference to the cheaper and more easily obtainable chlorid, nor is any statement made as to whether other developers than metol can be used. The dilute solution of thiosulfate prescribed causes a long delay in the fixing, and early in my own experiments I found that a 5% solution could be used. The elimination of all of the fixing agent is important, as mere traces of it will cause stains. The development is much slower than in the usual method, often requiring twenty minutes, although more rapid action is sometimes obtained. If the plate is immersed for some hours, the deposit may spread over the whole surface, becoming so dense as to obliterate the image and make the whole film opaque.

My investigations have been directed principally to determine whether other mercuric compounds than the bromid can be used, whether a stronger fixing bath is admissible and whether other developers than metol will give good results. Different types of plates have been tried, and potassium cyanid

has been used as fixing agent. Since the action depends essentially on the reduction of the mercury salt to the metallic state, it became of interest to study the action of the common organic developers on mercuric solutions. The experiments were carried out with a solution of 2.5 grm. of mercuric chlorid and 40 grm. of sodium sulfite (dry) in 500 c.c. of water. This solution deposits after a few days a crystalline mass, but still contains a notable amount of the mercuric compound. On the other hand, the mercuric bromid and sulfite solution gives no deposit after a considerable time. It is possible that the French investigators noted this fact, but, if so, they should have given the information in their paper. The studies of the effects of the common developers on the mercuric solution were made by adding solution of these developers to strong sulfite solution and mixing this with portions of the mercury solution. It was found that metol is most satisfactory. It is necessary, of course, to use only sulfite in connection with the development as carbonate or alkali would promptly precipitate an insoluble mercuric (or silver) compound. The oxalate developer is for the same reason out of the question. As different developers are influenced differently by the reaction of the solution, the conditions of the process prevent a complete study of their application.

Experiments were made with both mercuric bromid and mercuric chlorid solutions, in both cases with a large amount of sodium sulfite. The developer was also dissolved with sodium sulfite. Somewhat different effects can be obtained with same developer by using different proportions. The following are notes of some of the results:

	With mercuric bromid	*With mercuric chlorid*
Metol	Slight ppt. after some time	No ppt.
Hydroquinone	Slight ppt. soon	Slight ppt. soon
Glycin	Slight ppt. after 24 hours	No ppt.
Neol	No ppt.	No ppt.
Edinol	No ppt.	No ppt.
Pyrogallol	Ppt. soon	Ppt. soon
Pyrocatechol	No ppt.	No ppt.
Amidol	Ppt. soon	Ppt. soon
Paraminophenol hydrochlorid	Ppt. in sulfite solution	

From the above experiments it appears that metol is the most satisfactory of the commonly used developers for this procedure. Glycin acts similarly to metol, and a mixture of 1 part of the latter with 3 parts of the former makes a satisfactory developer, just as it does in regular development.

The deposit of metallic mercury is, of course, liable to disappear, as mercury is volatile even at comparatively low temperatures. An instance is noted below in which an image disappeared except as to a few scattered spots, although the plate was bound up as a lantern slide. The latent image, however, was still in full vigor, as was shown by re-development. It is likely that such re-development will be always possible, but this is an inconvenience, and it is worth while to determine whether some treatment cannot be given to the mercurial deposit which will make it permanent. It would seem possible to convert the metal into a sulfid, but immersion of the plate in solutions of alkali-metal sulfids does not give an appreciable effect. Exposure to vapor of iodin produces promptly a red iodid which may be considered permanent, but it is probable that if such a plate was used as a lantern slide, the heat would soon convert the mercuric iodid into its yellow form. It may be possible to convert the mercurial deposit into silver by immersing the plate in a solution of silver nitrate, but if this action occurs at all it is quite slow. Probably solutions of gold and platinum would act more promptly, but these are very expensive and their use can have but little interest either theoretically or practically. An attempt to iodize the image by immersing the plate in an alcoholic solution of iodin failed on account of the prompt softening of the film. The use of a water solution of iodin and potassium iodid is inadmissible, as the iodid would dissolve the mercuric iodid.

A way out of the difficulty of the volatile properties of the mercurial deposit would seem to be to use a less volatile yet reducible metal. Silver is the only one that comes into question. LL. & S. give a formula for a development with silver as follows:

A.	Sodium sulfite (dry)	18.0	grm.
	Silver nitrate	0.75	"
	Water	100.0	c.c.
B.	Sodium sulfite (dry)	2.0	grm.
	Paraphenylene-diamin	2.0	"
	Water	100.0	c.c.

The solutions are evidently modelled after those for the mercurial process and the same proportions (5 of A to 1 of B) are directed. In the original article, the silver nitrate is given as a volume of a 10% solution, but in the formula given above the amount is by weight.

I have had no success with this procedure. The developer promptly

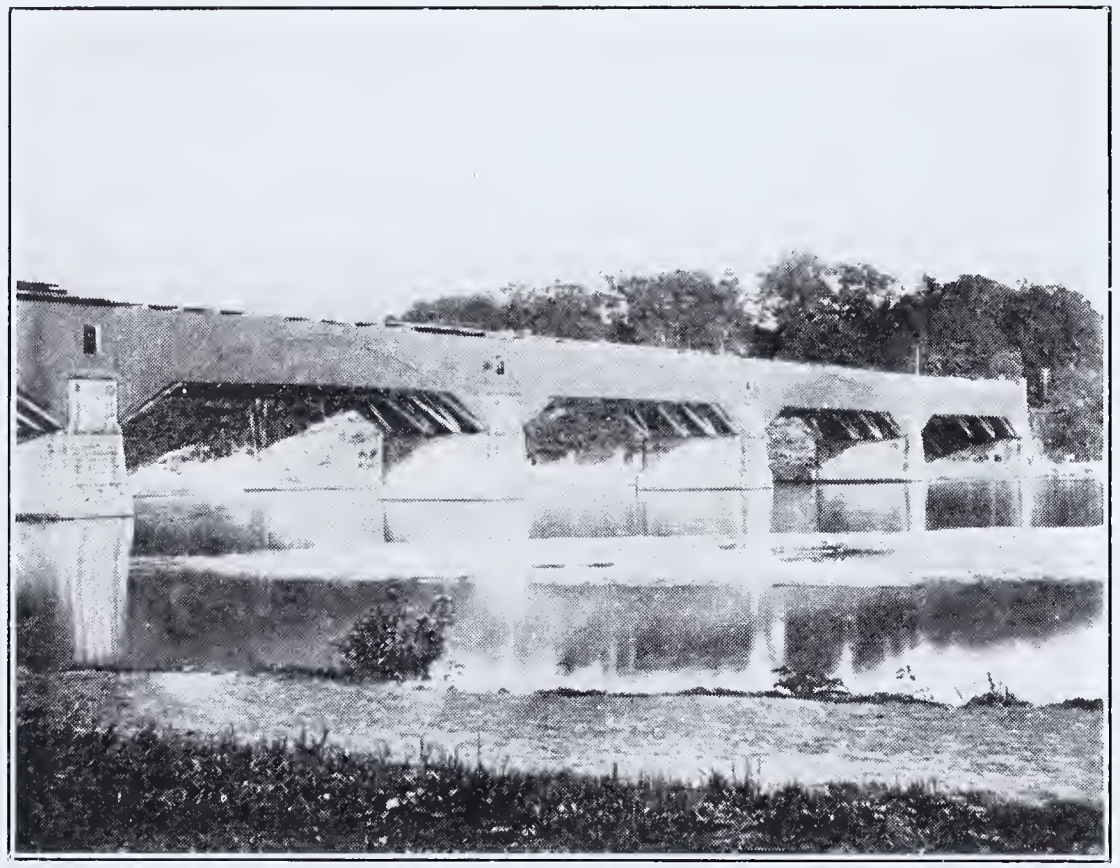
reduces the silver salt and no image can be obtained. LL. & S. state that some other developers, such as metol, can be used but are more rapid. In my own experiments all these reduce the silver so rapidly as to be of no value.

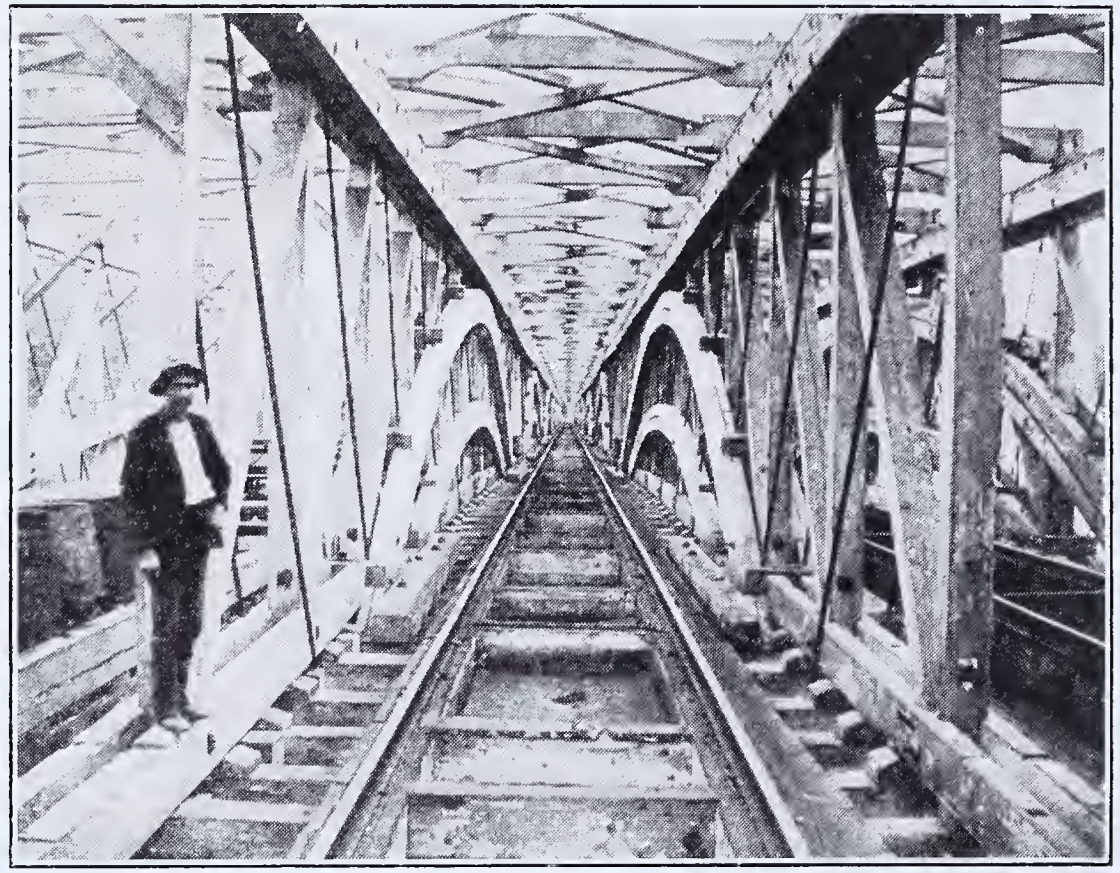
There are obviously many variations that can be made upon this procedure of post-fixation development, and certainly considerable theoretical interest attaches to the fact that the latent image abides in apparently full force after the silver halid is all removed so far as can be judged by the eye or by moderate magnification. It is, of course, not unlikely that a few specks of the sensitive emulsion remain undissolved even after long fixing, but these could not form the image complete in every detail that can be obtained when the procedure is carefully carried out. I have made excellent negatives and lantern slides by the method. Since the development can be carried out in daylight, the process can be conveniently watched and the density controlled. Potassium cyanid seems to destroy all traces of the latent image. Even with a 2% solution—which fixes rapidly and cleanly—a plate was obtained on which no development could be made.

The results of a large number of experiments indicate that the mercuric bromid solution with metol as the developer is the best method. Ordinary lantern slide plates give good results. Some of the special plates, such as the panchromatic, do not seem to respond. No trials were made with bromid paper, but LL. & S. state that it will also give good results. The mercuric solution keeps very well; the metol solution, of course, has not such a long life. The mixed solutions as a rule soon begin to be turbid. The fixing solution may be much stronger than LL. & S. state. Their limit is 2%, but I have obtained good results with the ordinary acid fixing bath, undiluted, both prepared with chrome alum and common alum. For ordinary lantern slides, however, a plain 10% solution of the thiosulfate is advisable, as these slides fix quite rapidly. Thorough washing is, of course, necessary both after fixing and after development. On several occasions when the development was stopped after only a weak image had appeared, the washing of the plate seemed to increase the density materially. The explanation of this has not been ascertained. It may be due to some reaction of the mineral ingredients of the tap water with the mercury, but this seems hardly likely.

The following rather remarkable instance of persistence of the latent image was observed by me. Some of the common metals of low fusing points emit at ordinary temperatures enough vapor to affect a sensitive plate after

a few days' exposure. Mercury, as might be expected, is especially active. A plate of well-amalgamated zinc was scraped and on it was laid a piece of the red-black paper in which the daylight films are usually wrapped. A stencil of the letter "M" had been cut out of the paper. On this was laid an ordinary (unexposed) lantern plate. The arrangement was made in the dark and left so for several weeks. The plate was then fixed and subsequently developed in the mercuric bromid solution. A well-marked "M" was obtained. It was, therefore, a plate exposed in the dark and developed in the light! The plate was mounted as a lantern slide. After being exhibited, it was put away for a couple of years with other slides, and it was found that all of the image had disappeared except a few spots. The slide was opened, and the plate immersed in the mercuric bromid mixture. In a few minutes the image was fully restored.





The First Bridge, 1834–1886

Progress of Demolition of the First Bridge

THE RECONSTRUCTION OF THE COLUMBIA BRIDGE, PHILADELPHIA

SAMUEL TOBIAS WAGNER

ONE of the most interesting developments in engineering is the replacement of structures after relatively long intervals of time with materials and design which are essentially different.

One of these which has been selected as an interesting type of this subject is what is known as the "Columbia Bridge" over the Schuylkill River, in the city of Philadelphia, where the changes in design were due to the ever-increasing size of motive power and rolling equipment in use on the railroads in America.

Originally built in wood in 1834, it was rebuilt in wrought iron in 1886, and finally in concrete in 1921.

The bridge is located in Fairmount Park, in the city of Philadelphia, over the Schuylkill River, between what is locally known as Belmont and Rockland, and carries the main line of the Philadelphia & Reading Railway between the Reading Terminal, Philadelphia, and Reading. Over it passes a very heavy traffic, both freight and passenger. All of the freight from the yards at Front and Noble Streets, from Broad and Callowhill, as well as the industries along the line, together with the joint business between Baltimore and the south and west, via the Baltimore & Ohio, the Philadelphia & Reading and the Central R. R. of New Jersey, to New York, and also a very heavy traffic from the industries lying along the Schuylkill and Delaware Rivers from Twenty-fourth and Chestnut Streets, Philadelphia, to Chester and Marcus Hook, pass over this bridge. At the present time it also carries the passenger trains of the Baltimore & Ohio Railroad from Chicago and St. Louis to New York, from Twenty-fourth and Chestnut Streets, Philadelphia, over the Philadelphia & Reading to a point near Bound Brook, N. J., thence over the Lehigh Valley Railroad to Newark, N. J., and from that point to New York over the Pennsylvania Railroad.

In all about 80 trains, every twenty-four hours, with an approximate tonnage of 12,000 tons, are handled over this portion of the main line, besides

numerous shifting movements to and from the Belmont Freight Yard which is located at the north end of the bridge.

It was largely due to the increase of traffic over the bridge which made its last replacement necessary. The weight of railroad traffic has increased largely of late years by increasing the tonnage of freight trains, followed by a corresponding increase in the weight and size of locomotives and cars. The old wrought iron truss bridge existing in 1917 was too light to allow the heavy locomotives to pass over it and its replacement was therefore necessary. The existence of old weak bridges on a railroad, until their replacement is required, calls for a reduction of speed in the passing trains, thus curtailing the amount of traffic. At the time of the last replacement of this bridge all trains were required to reduce speed to twenty miles per hour.

HISTORY

The Columbia Bridge was built in the year 1834, by the Canal Commissioners of the State of Pennsylvania, for the Philadelphia & Columbia Railroad, one of the earliest steam lines constructed in the State of Pennsylvania, and in fact in the United States. It was 1,050 feet long, divided into seven spans, and carried a double track railroad and a carriageway covering a total width of superstructure of 48 feet. It will be found that structures as wide as this in the early history of bridge building are very rare. It was built of white pine timber and owing to the careful manner in which it had been protected from the weather the bridge was as good when it was removed in August, 1886, as it was when first built in 1834.

This wooden bridge was the first railroad bridge of any considerable size constructed in the United States, and was the oldest wooden railroad bridge of any considerable size in existence when it was dismantled and removed in 1886 to make way for a wrought iron superstructure. When the wrought iron bridge was removed in 1921 to make way for a concrete arch bridge most of the wrought iron was in excellent physical condition. Only in a few of the upper lateral struts, affected by the locomotive fumes, was there any deterioration. The old Phoenix columns when cut apart showed a condition on the inside practically as good as when they were fabricated, although no paint had been applied in the thirty-five years of their existence.

The following quotation from an old description of the bridge may be of interest:

The Second Bridge, 1886–1920. Looking West from Up-stream Side

The Second Bridge

PROGRESS OF WORK ON THIRD BRIDGE

PROGRESS OF WORK ON THIRD BRIDGE

"Strange burdens have been borne across the old bridge during its half century of usefulness. Unnumbered thousands of foot passengers have tramped its floor and vehicles of every description have rolled through the dark, tunnel like structure. Not only have freight and passenger trains crossed the river upon its substantial arches, but even canal boats have been trundled across and brought into the very heart of the city. Until the construction of the Pennsylvania Railroad, numerous canal boats were built in detachable sections. Freight was loaded on these craft at Pittsburgh and other points, and the boats were taken by land and water to their destination. At the termination of the water routes these sections of the canal boats were detached and each one run upon a truck. They were then drawn from the water and placed upon railroad tracks over which they were conveyed to their destination. Freight often loaded upon boats in Pittsburgh was often unloaded from the same vessels in Market Street warehouses."

The above description refers to what was known as the State Works, from Pittsburgh to Philadelphia, embracing the western division of the Pennsylvania Canal from Pittsburgh to Johnstown; the Portage Railroad with its ten inclined planes over the mountains to Hollidaysburg; the middle division of the Pennsylvania Canal extending from Hollidaysburg to Columbia, Pa., and the Philadelphia & Columbia Railroad with its inclined plane at Columbia ascending from the Susquehanna River, thence by locomotives to the head of the inclined plane at Belmont, descending to the Schuylkill River where it crossed the Columbia Bridge and entered the city of Philadelphia via Pennsylvania Avenue—the route of the old Union Canal—to Broad Street, and thence turned into Market Street.

The canal boats were built in three or four sections. At Johnstown they were placed upon trucks and transported over the mountains to Hollidaysburg. At Columbia they were again placed upon trucks and followed the line of the State Road to destination, being delivered into warehouses along Market Street, Philadelphia, as far east as Second Street.

When the old State Road from Columbia to Philadelphia was purchased by the Pennsylvania Railroad, it dispensed with the inclined planes at Columbia and Philadelphia, and relocated those portions of the line, extending the eastern end to Thirty-second and Market Streets, where locomotives were taken off, string teams of horses substituted and the cars were drawn to the passenger station at Thirty-second and Market Streets, Philadelphia.

At Columbia, the inclined plane was dispensed with and the road relocated to a connection with the Pennsylvania Railroad Company's line at Locust Street.

The main line of the Philadelphia & Reading Railroad was opened for traffic in 1838 between the cities of Philadelphia and Reading, and in order to reach the center of the city of Philadelphia the railroad used a portion of the State Road, jointly with the State of Pennsylvania, from a point just north of the Columbia Bridge, to its terminal in the heart of the city. On acquiring certain sections of the State Road in 1851, the Philadelphia & Reading Railroad Company immediately renewed some portions of and strengthened the bridge, adapting it to the use of locomotives instead of horses for hauling trains.

When the wooden trusses were removed in 1886, the bridge was rebuilt under contract with the Phoenix Bridge Company, with a bridge with seven double track, wrought iron through truss, Phoenix column design, with spans about 150 ft. each, and carried a sidewalk on the down-stream side. There being no further use for the driveway it was abandoned. The old piers, although much too long, were used for the new bridge. The bridge built at this time was of Pratt trusses, with Phoenix column compression members, and a floor suspended from the lower chord pins by bent loop hangers. With the increase of the loads over this structure, these loop hangers were the first points to show distress and were strengthened previously to the removal of the structure.

On account of the overstrained condition of the structure, it was decided to replace it with a modern bridge consisting of concrete arches, carrying four railroad tracks instead of two, with a sidewalk on the up-stream side, and to build a bridge which would be worthy of its location in Fairmount Park, and a memorial to the original structure with such an historical record.

THE NEW STRUCTURE

The plans provided for a slight change in the location of the bridge so as to allow two tracks of the new structure to be built on the up-stream side of the old wrought iron bridge, in order that railroad traffic could be carried on the old bridge until two tracks were ready on the new structure, then remove the old structure and build the remaining two tracks on the down-stream side.

After submission of the plans to the Water Supply Commission of Pennsyl-

vania, the Public Service Commission of Pennsylvania, the War Department and the Fairmount Park Commission (and by them submitted to the Philadelphia Art Jury), the plans were finally approved by all and the work was placed under contract on July 6, 1917.

The bridge as reconstructed consists of eight concrete arch spans, two over the east and west drives in Fairmount Park, each with 70 foot clear spans, and six arches over the Schuylkill River with clear spans of 100 ft., 2⅝ ins. each, all measurements being taken at the springing lines of the arches and at right angles to the faces of the piers. Parts of the old abutments were used for wing walls, but the abutments themselves and all of the old piers in the river were removed. The locations of the new piers were designed so as to allow the up-stream half of the new bridge to be built without interfering with traffic on the old spans and their piers.

In order to provide the proper rise for the new arches and give the required distance from top of rail to the intrados of the arches, and maintain the necessary clearance over the Park Drives, it was necessary to raise the elevation of the tracks over the bridge about three ft. above the level on the old bridge and to revise the approach grades.

THE DESIGN

The design provides for four tracks, 13 ft. center to center, with a footwalk four ft. wide on the up-stream side, separated from the nearest track by an iron picket fence six ft. high, and with concrete railings on each line of coping. Vitrified clay ducts were built in each coping for electric light, signal, telegraph and telephone wires.

The centre lines of the piers are placed parallel to the direction of the current in the river, and make an angle of 65° 0′ 41″ with the centre line of the bridge. The total length of the bridge between faces of abutments is 971 ft. 3⅞ ins., and the total width under coping is 57 ft. 6 ins.

Each arch is the segment of a true circle, with a rise of 20 ft. 6 ins., the radii of the larger arches being 124 ft. 9 ins. and 71 ft. 5½ ins., and the smaller 76 ft. 3¾ ins. and 40 ft. 1½ ins., giving a crown thickness of 5 ft. and 4 ft. respectively. Abutment piers located in the river channel are 28 ft. wide at the springing line. The two piers at the short spans over the driveways are 20 ft. wide and the intermediate piers 16 ft. wide. The design permits the turning of the two large arches and the removal of the steel centering in one

operation, the abutment piers providing for the stability of the concrete arches as placed during construction. All of the piers have circular ends and are battered ½ in. per foot on all faces.

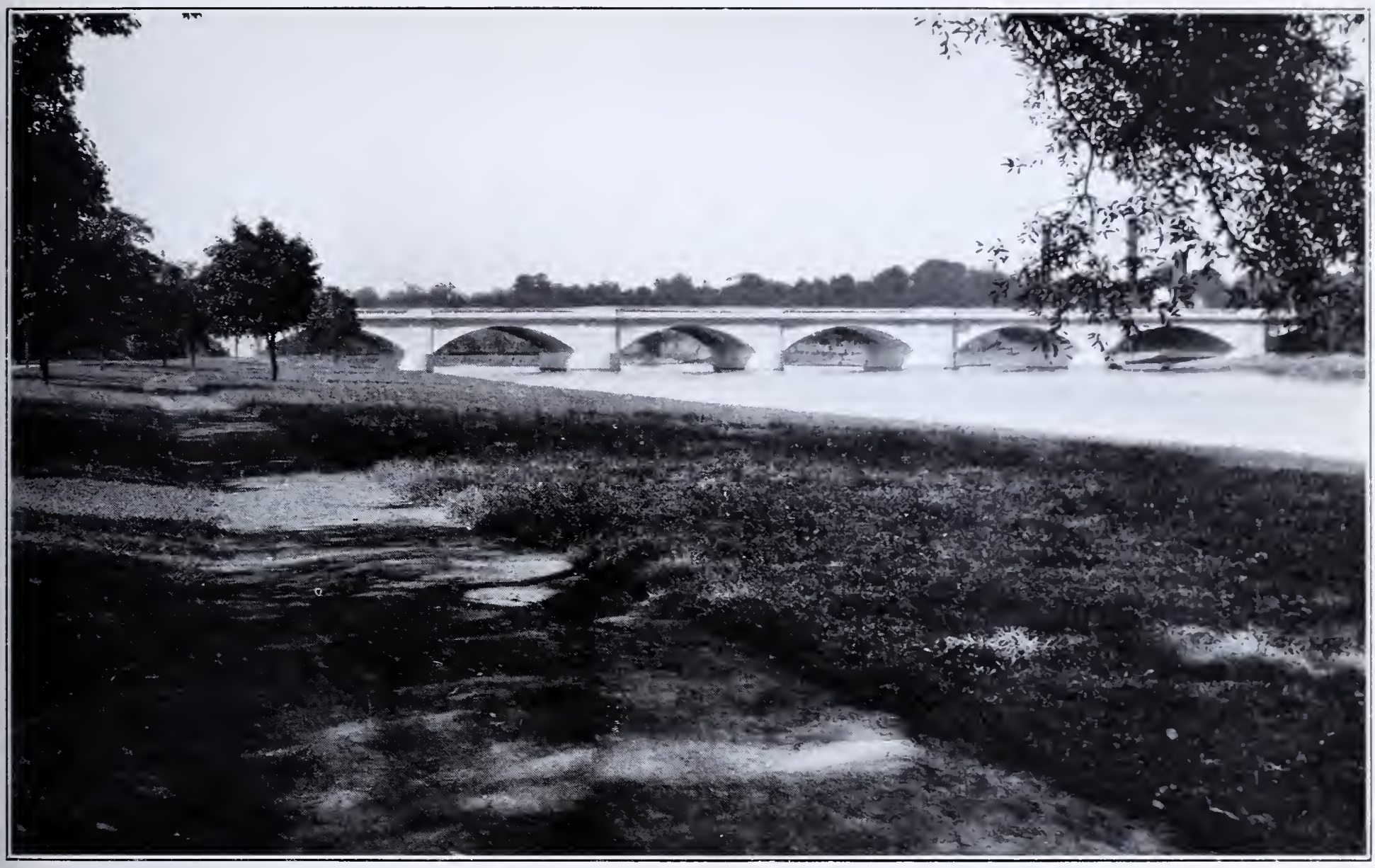
The design of the arches is such that the only stress carried by the concrete is compression. Steel reinforcement was used only to provide for stresses due to change in temperature in the piers, arch rings, spandrel walls, copings and railings. In all cases this reinforcement was placed 3 ins. back from the face of the concrete. The reinforcement in the neat work of the piers consisted of ½ in. rods spaced 3 ft. centres vertically and 2 ft. horizontally. In the arch rings, extrados and intrados it consisted of ⅝ in. rods spaced 10 ins. centre to centre parallel with the centre line, and ½ in. rods spaced 3 ft. centres parallel to the faces of the piers. In the back of the spandrel walls ⅝ in. rods were placed 1 foot centres. Every alternate rod extends the full height of the spandrel walls and into the arch ring for a distance of 2 ft. This arrangement provides a bond between the arch ring, the spandrel wall and the coping, the long rods also extending into the coping.

For construction purposes the large arch rings were divided into seven sections and the small into five. The construction joints in the arch rings were carried through the spandrel wall, the coping and the balustrade, after which the top of the joint through the sidewalk and the coping was filled with elastic waterproofing cement to prevent any water passing through the joint and down the face of the spandrel wall.

At the piers the construction joint was made where the post and balustrade met, and in order to prevent cracking of the balustrade No. 16 sheet zinc was placed between the joint under the balustrade and the post so that the balustrade concrete between these two points is free to move with changes of temperature.

METHOD OF CONSTRUCTION

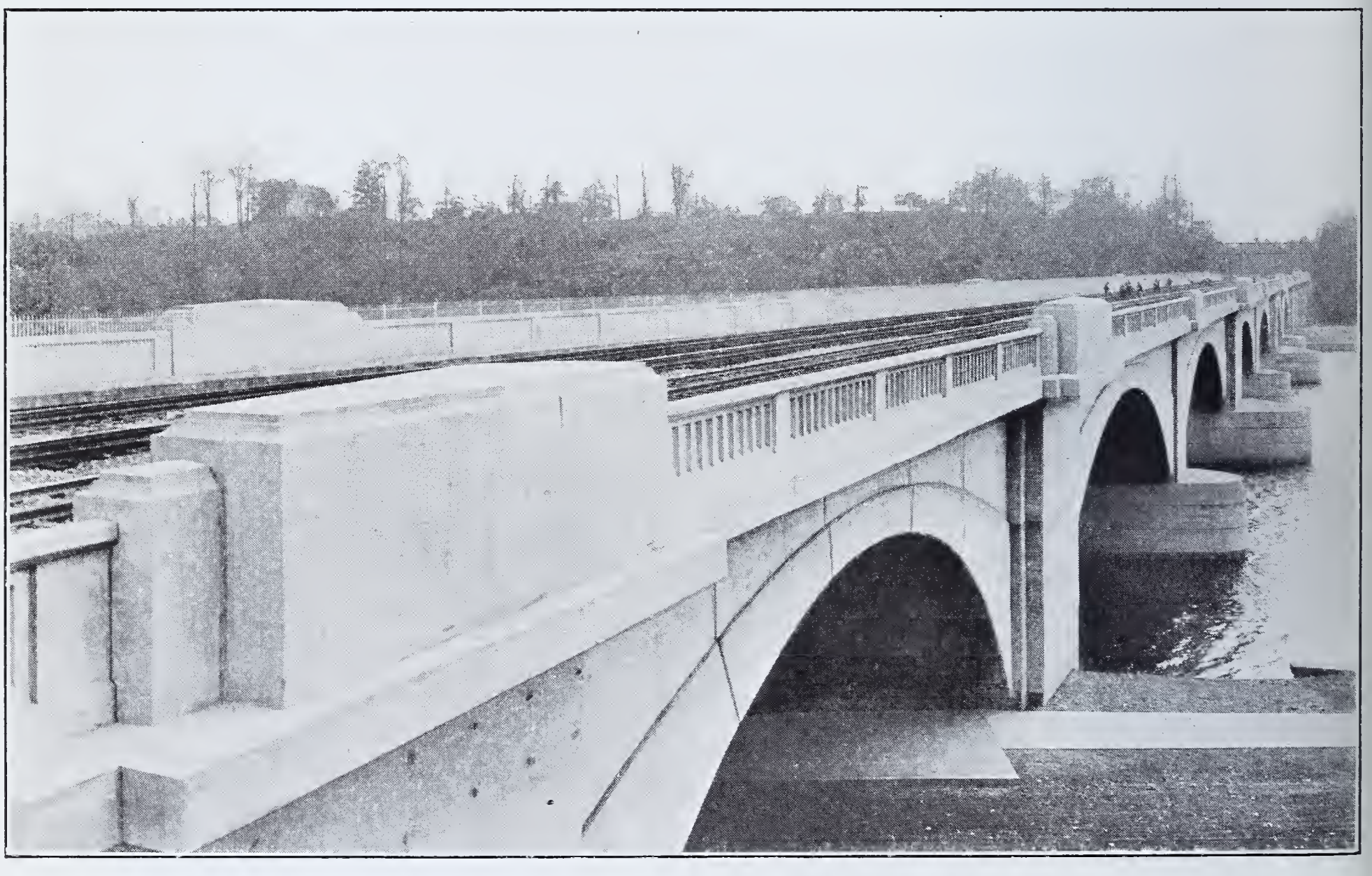
The work of construction was started in July, 1917. Excavation was started at the north abutment. Wood was used for all framework and sheeting. The work was carried on for two river foundations at one time and progressed from the shores toward the centre of the river with the greatest depth of foundation below normal water level of 23 ft. The most troublesome foundation to excavate was Pier No. 1 on the east side of the river, the material removed being large stone, a cubic foot and greater in volume, similar to the



The Third Bridge. Up-stream Side, Looking West

The Third Bridge. Up-stream Side, Looking West

The Third Bridge. Looking West



The Third Bridge

material composing the entire area of the bank between the river and the Park driveway. This material was placed along the river banks many years before when the driveway was widened by the removal of part of the rock bluff immediately adjacent. The water from the river reached through the excavation so that a puddle was necessary along the river bank for a distance of 300 ft. in order to stop the flow. The bottom of this foundation was 20 ft. below normal water level. The foundations of all piers and abutments were on bed rock.

Wooden forms were used for all neat concrete, the lumber being tongue and grooved, surfaced two sides and 1½ ins. thick. Wooden centres were used for the 70 ft. spans over the driveways, and steel centres for the river spans, a sufficient number being provided for turning two arches at a time. The forms for the umbrella sections over the piers were supported in some cases by the steel centering and in other cases were built up and supported from the foundation offset of the piers.

Floating derricks were used for handling the steel centres, the cofferdam construction, the excavation from the river piers and the puddling for the outside of the cofferdams. The material for the puddle was dredged from the river channel, up and down stream from the bridge site.

WATERPROOFING

It is generally recognized by engineers that the life of concrete structures will be largely prolonged by proper drainage, so that water falling upon the structure will be removed as promptly as possible and be prevented from entering the construction joints, thereby causing disintegration of the concrete. Allowing water to seep through a structure produces unsightly staining of the surface and gives a very bad appearance. In the present case the structure is over two of the principal drives in Fairmount Park, which are thronged with pleasure seekers, and over the river, which in summer is filled with pleasure craft. The bridge is also but a few hundred feet below the finish of the 1½ miles regatta course on the Schuylkill River. For all of these reasons a special effort was made to build a structure which should be as uniform in appearance as possible. Consequently the backs of all arches and spandrel walls were covered with a waterproofing membrane consisting of three layers of an asphalt saturated cotton fabric fastened to the concrete and each layer mopped with melted asphalt, thus forming a waterproofing blanket. This was pro-

tected from injury by being covered with a layer of 1-2-4 concrete, 2½ ins. thick, reinforced with No. 12 electrically welded wire cloth, with 4 in. by 4 in. mesh. All of the filling over the arches to the subgrade of the tracks consisted of one and two men stone packing. Drainage was provided at each pier through an 8 in. cast iron pipe with the discharge end of the pipe placed below the normal water level in the river. Ordinarily such discharge pipes empty into the air below the structure and any soiled water from them is liable to be blown against and disfigure the structure. Masonry manholes leading from the upper ends of the pipes were built up to the level of the tracks and provided with cast iron covers so that the drain pipes (and the openings which were provided in the side of the manholes) can be cleaned. The outlet of the drains being below normal water surface are not visible and they should not be stopped by ice forming in the pipes except in extremely cold weather when an unusual thickness of ice forms in the river.

The specifications for the waterproofing above referred to were dated 1916. Considerable improvement in this art has been made since that date. They provided for a fabric of woven cotton containing in its raw state no oils of any kind. It was thoroughly saturated with a natural bitumen consisting of an asphalt with the following properties:

A fluxed natural asphalt or an asphalt prepared by the careful distillation of an asphaltic petroleum. In its refined state not less than 98% of bitumen soluble in cold carbon disulfid. The remaining ingredients such as not to exert an injurious effect on the work. When 20 grm. were heated for five hours at a temp. of 325° F. in a tin box 2½ ins. in diameter, it did not lose more than 2% by weight, nor was the penetration at 77° F. after such heating less than one-half the original penetration. The melting point was between 150° and 190° F. A briquette of solid bitumen of cross section of 1 sq. cm. showed a ductility of at least 3 centimeters at 40° F. At a temp. of 77° F. the ductility was not less than 20 centimeters.

No oils, petroleum residues or other petroleum solvents were used to liquefy the asphalt used as a saturant, but it was produced entirely by heat and pressure.

The fabric was elastic, having a stretch in any direction of at least 10% without fracture. It was proof against puncture by a stick with a base 1 in. square weighted to 90 lbs. It was flexible at all temperatures between 0° and 250° F.

The fabric was laid on the concrete in the following manner: The surface of the concrete was thoroughly cleaned of dust, dirt, loose particles and all grease and painted with two coats of approved asphalt diluted with gasoline. The first coat proportioned to give a brownish tint, the second to have a larger amount of asphalt. Over the concrete so prepared a mopping of hot asphalt similar to that described above was applied. The mopping not to spread over more than 1 sq. yd. of surface. Into this mopping is laid the first layer of the fabric. A second mopping of asphalt then applied followed by the second layer of fabric which was applied lapping the first strip two-thirds of its width plus 2 ins., and so on until the top layer of fabric was covered with a mopping of asphalt.

Work of this kind requires the most careful attention to detail, specially in flashing the edges of the membrane so as to seal against the entrance of all water.

Where the membrane finished against a face of concrete the sealing was effected by means of a pocket of elastic fibrous cement of such a composition as to remain plastic at all temperatures and so arranged that it cannot run at extremely high temperatures.

The work of waterproofing the up-stream side of the bridge was started June 16, 1919, and completed by working in three separate sections on December 31, 1919. The actual working days consumed was fifty-two. On the down-stream portion work was begun September 8, 1920, and in five separate operations was completed June 15, 1921. The total time consumed on the down-stream side was seventy-seven days.

CONCRETE

As has been stated, the concrete used was mixed in the proportions of 1 part Portland cement, 2 parts fine aggregate and 4 parts coarse aggregate. The cement was furnished by the Railway Company to the contractor and was in accordance with the Standard Specifications of the American Society for Testing Materials. The fine aggregate was sand, generally dredged from the Delaware River. The coarse aggregate was generally crushed pebbles, dredged from the Delaware River, and was the entire product of the crusher passing through the screens from ¼ in. up to and including 1½ ins. For the coping and railings the maximum size was ¾ in. The consistency was such that when dumped in place it would not require tamping but was not wet enough to cause

the mortar to separate from the coarse aggregate. While being placed in the forms it was very carefully worked so that the coarse aggregate was kept away from the surfaces. After the last form had been removed from the work, all exposed surfaces, except the intradoses of the arches, were rubbed to remove the form marks. For this purpose the contractors used a Berg concrete surfacing machine operated by electric power.

All of the foundation concrete as well as the neat work to the springing line was placed to the full width of the new bridge at the time the up-stream side was built, except at the north and south abutments and at piers Nos. 6 and 7, where the masonry of the old truss bridge prevented the work being done.

The largest volume of concrete placed at one time without interruption was 525 cubic yards. This required sixteen hours and twenty-five minutes to place and was in a portion of the umbrella of pier No. 5.

Most of the concrete was mixed at the north end of the bridge and transported by narrow gauge cars, attached to a cable and operated by a hoisting engine at the same end, the movements being controlled by electric bells. The narrow gauge track was placed at the side of the old truss bridge and supported by the lower chord members. Two one-yard side dump cars were attached to the cable, and the concrete chuted from the cars through spouts into the forms.

The concrete mixing plant consisted of two one-yard mixers operated by steam and fed from an overhead storage bin by gravity. The sand and crushed gravel were "clam-shelled" into the bin from storage piles. From the mixers the concrete was elevated and chuted into a hopper over the narrow gauge track that delivered it to the dump cars. Embedded stone was used below the springing line in all foundations, piers, and abutments. Practically all of this stone came from the piers and abutments of the old structure.

DATES OF PROGRESS OF THE WORK

The work of excavation for the north portion of the north abutment was started in July, 1917, and the entire excavation for the up-stream portion of the new bridge was completed in September, 1919.

The placing of the concrete followed the excavation very closely and all of the foundations for the up-stream portion of the bridge were concreted on September 11, 1919.

The concrete in the last arch ring was poured on November 4, 1919, and all of the concrete for the up-stream half was completed on November 22, 1919.

Railroad traffic was directed to the two tracks on the up-stream portion of the bridge on March 24, 1920, the first train passing over it being a north-bound train about 11 A. M.

The old wrought iron truss bridge was removed between March 29 and August 6, 1920, the work of removal being started from the south end of the bridge and progressing toward the north.

After the removal of the old bridge the work of the removal of the masonry in the south abutment was started on April 20, 1920, and the removal of all the masonry of the old bridge was completed on January 19, 1921, old pier No. 3 being the last to be removed.

The concrete was placed in the south abutment, down-stream side, on May 20, 1920, and all of the concrete was placed in the entire bridge on July 13, 1921. Traffic on the railroad was placed on the entire four tracks on October 11, 1921.

The work of reconstruction was carried on between 1917 and 1921 during part of which time the World's War was in progress and great difficulty was experienced in obtaining men and materials. The progress of the work was therefore not as rapid as was desired. A schedule of the estimated and actual dates of starting and completing excavation, form work, and placing concrete, was used so that all of the supervisory forces were kept informed as to where the work should be pushed.

The largest number of men employed daily was 160, while during cold weather the average was about 60.

REMOVING OLD BRIDGE

A separate contract was made for the removal of the old wrought iron bridge. On account of its being of wrought iron the old material had a special value as scrap, and as the design was entirely obsolete and entirely too light for modern traffic, it was scrapped. The contractor separated and classified the various kinds of material as it was cut apart. Being of the old Phoenix column type with cast iron connections, very limited cutting was required in the field to remove the old structure. To accomplish the work of dismantling the old bridge, the contractor placed bents under the panel points of each span.

Those over the driveways were placed on the ground, those for the spans over the river were on piles driven and used as the posts of the bents.

OLD FOUNDATIONS

The old masonry piers built in 1834 rested on hewn timbers of large cross section, some of the pieces being 16 ins. by 23 ins., and consisted of several layers at right angles to each other. This mass of timber rested on a thin layer of gravel overlying the bedrock.

Upon the removal of the old piers in the reconstruction of the bridge, the old timber was found to be in as sound condition as it was when laid in 1834.

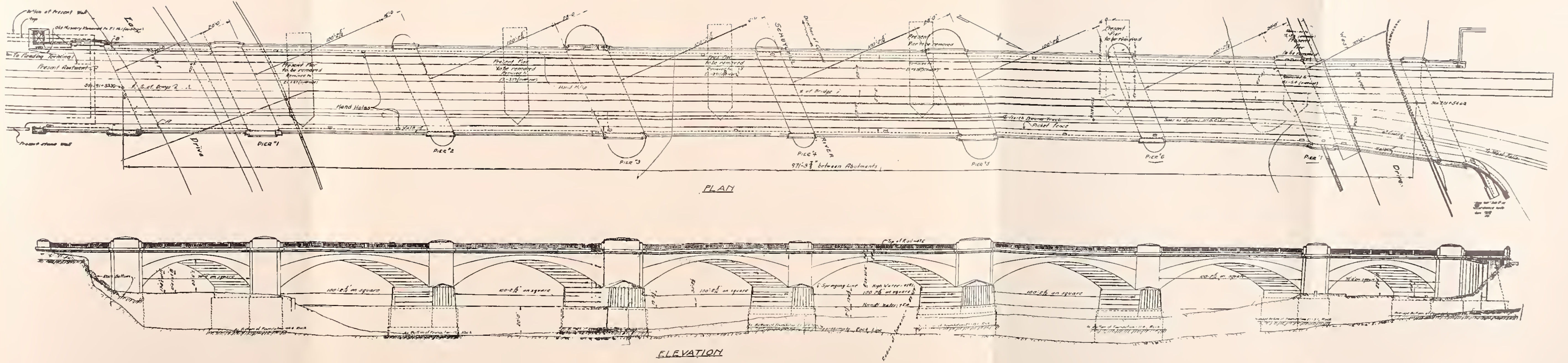
QUANTITIES

The magnitude of a piece of engineering work is often well shown by a list of the quantities of materials entering into it and the amount of excavation required. The following figures are therefore appended for this purpose:

Dry excavation	3,961 cu. yds.
Excavation in water	10,915 " "
Old masonry removed, dry	7,396 " "
Old masonry removed, in water	2,514 " "
Concrete in foundations	11,371 " "
Concrete above foundations	32,551 " "
Terra-cotta ducts	2,114 lin. ft.
Membrane waterproofing	66,290 sq. ft.
Fibrous cement	2,060 lbs.
¼-in. sheet asphalt for joints	2,354 sq. ft.
Iron picket fence	1,120 lin. ft.
Steel reinforcement in concrete	379,156 lbs.
Electric welded wire cloth	70,059 sq. ft.
Drain pipes, 4 in., 6 in., 8 in.	678 lin. ft.

CONTRACTS

The contracts for the grading and the construction of the masonry, including the foundations, were placed with Messrs. Seeds & Derham, of Philadelphia. The waterproofing was done under a contract with the Minwax Company of New York. The removal of the old wrought iron superstructure was by Henry Hitner & Sons, Philadelphia.



Reconstruction of Columbia Bridge Over Schuylkill River. Scale 1" = 47'.
Samuel T. Wagner, *Chief Engineer.*

Transactions, Wagner Free Institute of Science of Philadelphia.
Volume X, 1923.

PERSONNEL

All work was done by the Philadelphia & Reading Railway Company, Mr. Agnew T. Dice, President; Mr. Charles H. Ewing, Vice-President; Mr. Samuel T. Wagner, M. Am. Soc. C. E., Chief Engineer; Mr. Clark Dillenbeck, M. Am. Soc. C. E., Assistant Chief Engineer; Mr. P. S. Baker, M. Am. Soc. C. E., Engineer, Bridges and Buildings.

The late Mr. Edwin Chamberlain was Assistant Engineer in general charge of construction, and Mr. Harry B. Glisson, Assistant Engineer in charge of the forces at work in the field. The forces under Mr. Glisson consisted of Mr. W. Kirk Wyatt, Transitman; Mr. Jacob G. Sweed, Levelman; Mr. Carl O. Bauer, Rodman. The inspection force consisted of Mr. J. B. Rieg, assisted by Mr. Thomas W. Rorer.

AN INQUIRY INTO THE ORIGIN AND RELATIONSHIP OF CERTAIN NORTH AMERICAN SONG BIRDS

SPENCER TROTTER

1

THE science of zoology is fundamentally concerned with the problem of the classification of animals, just as botany is concerned with the classification of plants. In this it is altogether different from those branches of learning which have for their object a study of the structure and life histories of organisms—biology in its generally accepted sense, morphology, ecology, and animal behavior. In other words, zoology is, in the main, taxonomic. It embodies a certain attitude of mind toward the logical arrangement of animal forms in relation to one another. These relationships are, however, based on structure, so that morphology is very truly a province of zoology, and in this more comprehensive view ecology and animal behavior may likewise be considered as provinces of the larger science of zoology.

The several groups into which the various forms of animals naturally fall, based on the structural likenesses and differences which pertain to their peculiar mode of development in each group, constitute the more primitive *types* or *phyla*. These types are again subdivided, according to lesser deviations of structure, into *classes*, and it is these latter divisions that embody our familiar conceptions of broad animal differences—the common, every-day fact of fish, flesh, and fowl. Each one of these great primary divisions of animal life has become a special object of study and research within the larger domain of zoology.

Comparatively few persons of the many now interested in the study of birds are much concerned about the taxonomic value of the characters which unite a number of genera that constitute a given family, but few probably realize just what the distinctive characters are in any group of birds, or just what they mean. It is the *species* that counts with most of us, the particular mode of life, nesting habits, song, distribution, form and color pattern of a particular kind of bird. We know it to be related to some other kind of bird, but how? on what taxonomic basis? A species is an assemblage of individuals

that are practically all alike within a given area of country, and occurring outside of this area may be slightly changed, but still recognizable as a single species. To these minor variations we ascribe geographical or habitat influences and regard them as sub-species or geographical races, giving to the whole group of individuals, thus differing among themselves, a trinomial designation. More pronounced differences which extend beyond mere slight external variations into nervous reactions, as habits, song, nest-building, and peculiarities of habitat, constitute a distinct cleavage among individuals, and the mind, taking note of these facts, regards one group of individuals thus differing from another group as constituting two or more different species.

Among the host of individuals we of course note that though differing in many ways, certain groups of these different species are more nearly related to one another than they are to other groups. Hence we unite these more closely related species into a group that we call a *genus*, which we regard as a still further differentiation of individuals from others of a group of like value. The genus stands in our minds for a bond of relationship among species of like nature and implies a common ancestry for the different related species that is less remote in geological history than is the common ancestry of several related genera.

Now all this is a matter of our judgment in what is revealed to us through our senses. In all this, too, there is but one real, tangible fact, the individual; it we can see, handle, measure, study: its qualities are revealed to us through our senses. But when we come to take note of the vast assemblage of individuals and the varying degrees of difference which they present among themselves, we are entering the domain of judgment in which we attempt to construct a scheme expressing our ideas of such relationships. It is well to bear in mind, then, that both the species and the genus are purely psychological, existent only in our minds, not tangible entities like the individual.

With these close relationships of individuals as exhibited in our conceptions of species and genus, we cannot go very far astray, the differences and likenesses are still tangible. But when we seek to express our ideas of the relationships between genera and attempt to formulate a scheme that will group certain genera together under the general term "family," separating them, in our conception, from other genera which would fall into a different group of like value, we are spreading out, getting further away from the realm of our senses into the realm of judgment. It is an axiom of psychology that

we are never deluded by our senses, but are peculiarly subject to delusions of judgment. In a given lot of birds you may see very clearly the close relationships existing among individuals that force one to regard them as constituting a species, and you may take a still larger number of individuals of several species that show marks of close relationship and express your idea of this in the term "genus." You cannot, however, get the same pronounced characteristics when you come to group genera together into respective families, because you are spreading out, trying to get a lot of quite diverse individuals on a common footing, by what, in your judgment, should constitute such an assemblage. The same is more or less true also of the larger groups or "orders" of birds, but when we reach the class Aves as a whole, we find certain strongly marked primitive characters which at once separate this group of beings from all other vertebrates. Modern birds are peculiarly uniform in their general characteristics and this constitutes a great difficulty that the student finds in attempting to classify or divide birds up among themselves. They are modified and specialized reptiloids of comparatively late geological origin and their peculiar environment has imposed uniform structural features. Particularly is this the case with the latest and in some ways the most highly evolved section of the class, the Passerines. These were scarcely evolved as a distinct type before the early Tertiary, probably not until the Oligocene, and the type has not had time to differentiate very much within itself as have the more ancient forms of birds. A real difficulty presents itself when we come to define certain of these Passerine families on a purely natural basis, especially among the song birds, the suborder Acromyodian Passeres, or Oscines. In these families there are certain aberrant or outlying types, which quite clearly indicate the difficulty I speak of, and prevent us from framing in our minds any hard and fast lines of separation between one family and another.

The existing classification of Passerines is the result of long and detailed study by eminent ornithologists, who one and all have declared that much was left to be desired. Particularly are they in doubt as to the limits of certain families—the haziness of frontiers between one family and another. Dr. Coues in the "Key" says: "I know of no character that will relegate the Bobolink and Cowbird to *Icteridæ* rather than to *Fringillidæ*, in the current acceptation of these terms; and *Dolichonyx oryzivorus* is curiously similar in some respects to *Ammodramus caudacutus*."* We all know how the early ornitholo-

* "Key to North American Birds," Fifth edition, Revised, 1903, 464.

gists regarded these birds as "buntings." Like the finches, they have nine primaries with angulation of commissure, but the bill is not as conirostral, though more markedly so in *Dolichonyx* and *Molothrus* than in the rest of the *Icteridæ*. On the other hand, the Meadow Larks (*Sturnella*) approach quite closely to the old world *Sturnidæ* or Starlings. Again, in speaking of the family *Paridæ*, the Titmouses, Coues says: "they are hard to distinguish technically from Jays; but all our Jays are much over 7.00 inches long."* Of the Waxwings, *Bombycillidæ*, this writer says that it appears to be "an arbitrary and unnatural association of a few genera that agree in some particulars, but are widely different in others." Ridgway regards *Tanagridæ* as "a more or less artificial group" and would separate *Euphonia* and allied genera as a "distinctive family." I think it was the ornithologist Sundeval who spoke of Tanagers as "fruit-eating finches." Ridgway says in another place, "The absence of obvious rictal bristles is the only external character that I am able to discover which will serve to distinguish the *Icteridæ*, as a group, from the *Fringillidæ*." To the *Sturnidæ* and *Ploceidæ* they are likewise closely allied. As to the *Mniotiltidæ*, Ridgway quotes Dr. R. Bowdler Sharpe, the late eminent British ornithologist, to the effect that in "forecasting a 'readjustment of the family, which must inevitably take place some day,' suggests that *Setophaga* and its allies will probably be considered to be Flycatchers (*Muscicapidæ*) rather than Warblers, *Helminthophaga* and *Helminthotherus* will very probably prove to be Wrens, *Icteria* to be aberrant Vireonine form; while *Granatellus* will be placed with the Tanagers."†

I cite these few cases to show how very uncertain are the frontiers between many Passerine families. We all know how the Thrashers have been bunted from pillar to post—thrushes, wrens, mocking thrushes and so forth. They are possibly more closely related to an Oriental group, the Babbling Thrushes (*Timeliidæ*), than we might at first suppose, as Newton has remarked in his "Dictionary of Birds." They have the same character of short, rounded wing and a somewhat similar nervous vocal mechanism. In short the family groups of the Passerines represent a decidedly artificial arrangement. In our present state of knowledge it is probably the best that can be done and I, for one, realize the debt we owe to the taxonomic ornithologist, but we cannot fail to see that such families as *Icteridæ*, *Mniotiltidæ*, *Sylviidæ*, *Mimiidæ*, *Bombycillidæ*, and others are far from natural associations of species.

* Ibid., 267. † "The Birds of North and Middle America," Part II, U. S. Nat. Museum, 1902.

The taxonomic value of the characters that define the genera of Colubrine snakes as compared with the characters that separate the families of Oscine Passerine birds is of interest in its bearing on the relative value of diagnostic characters in general. We are here comparing an *order* of more or less recently specialized forms of birds with a *family* of rather late evolution among reptiles. In the first place, the order Passeres is defined among all other birds by the structure of the foot and the perching mechanism. The family *Colubridæ* is defined by the maxillary bones, which are horizontal and not excavated, and the fact that the upper jaw bears solid teeth only. These two sets of taxonomic characters are of fairly equal value, both being of a deeper structural nature than the differences which pertain to the subdivisions of the two groups in question, and yet in birds the characters serve to differentiate an order, in serpents, a family. The genera of Colubrine snakes are diagnosed from one another by more or less purely superficial characters and the same is true of the families of Passerine birds. Thus the Colubrine genera fall into certain well-marked groups based on the characters of the rostral plate, the presence or absence of a split anal plate, and the keeled or non-keeled character of the dorsal scales. The families of Oscine Passerine birds, likewise, fall into groups based on the structure of the tarsal envelope, the notching and form of the bill, the number of primaries, and the character of the nasal feathers. In both instances the subdivisions are based on purely *epidermal* features of much the same value, and yet one entire group is regarded as an *order*, and the other as a *family*. The zoological point of view would reconcile this possible discrepancy by regarding the group of birds in question as *Passeridæ*, a family of a larger order, or conversely by looking upon these snakes as forming an order *Colubres*. Fortunately it is not a matter for the ornithologist to settle, but at the same time it is quite helpful to look at our particular study from another viewpoint.

It is with the relationship of the subordinate groups among themselves, within the larger group, no matter what title we may apply to it, that this paper has to do. In conformity with this idea I have selected what might be called the *outlying types* of certain families of North American Oscines, contrasting these with genera that contain a relatively large number of species that might be said to represent a central group of more or less typical forms which embody our notion of the family characters. In the wood warblers (*Mniotiltidæ*), for example, *Dendroica*, *Geothylpis*, and *Helminthophaga* might be regarded as more or less typically Mniotiltine, while *Icteria*, the Setophagine

BIRDS

ORDER PASSERES

Families of Oscine Passerine Birds

Foot structure insessorial.

Tail feathers—12. Primaries—9–10

A. Hinder edge of tarsus *not* compressed; rounded and scutellate, like anterior edge. Claw of hallux long. Developed primaries—9.
   Family—*Alaudidæ*—larks.
B. Hinder edge of tarsus compressed, undivided. Primaries—9.
   Family—*Icteridæ*—orioles, grackles, bobolink, red-wing, etc.
   Family—*Fringillidæ*—finches, sparrows, buntings, grosbeaks, etc.
   Family—*Tanagridæ*—tanagers.
   Family—*Mniotiltidæ*—wood warblers, yellowthroats.
   Family—*Motacillidæ*—pipits, wagtails.
   B[1]. Bill fissirostral; wing acute. Primaries—9.
   Family—*Hirundinidæ*—swallows.
C. Primaries—10. Tarsus scutellate and *not* longer than middle toe and claw.
   Family—*Bombycillidæ*—waxwings, cedar bird.
   C[1]. Tarsus longer than middle toe and claw. Bill notched and hooked.
   Family—*Laniidæ*—shrikes.
   Family—*Vireonidæ*—vireos.
   Bill decurved. Tail feathers stiff and pointed.
   Family—*Certhiidæ*—creepers.
   C[2]. Bill with stiff feathers covering nostrils; not notched.
   Family—*Corvidæ*—crows, jays, etc.
   Family—*Paridæ*—titmouses, etc.
   C[3]. Nasal feathers erect, not covering nostrils.
   Family—*Troglodytidæ*—wrens.
D. Tarsus "booted"—not scutellate.
   Family—*Turdidæ*—thrushes.
   Family—*Sylviidæ*—kinglets.

SNAKES

FAMILY COLUBRIDÆ

Genera of Colubrine Snakes

Horizontal maxillary *not* excavated.

Upper jaw with solid teeth only.

A. Teeth *not* grooved nor scales keeled. Anal plate bifid. Internasals, 2. Head not distinct from body.
   Genus—*Carphophiops*—ground-snake.
   Genus—*Abastor*—hoop-snake.
   Genus—*Virginia*.
   Genus—*Farancia*—horn-snake.
B. Head more or less distinct from body. Rostral plate normal, not recurved nor keeled. Anal plate divided. Dorsal scales more or less keeled.
   Genus—*Regina*.
   Genus—*Natrix*—water snake.
   Genus—*Callopeltis*—corn snake, fox snake, pilot snake, chicken snake.
   Genus—*Storeria*—red-bellied snake.
   Genus—*Haldea*—brown snake.
   Genus—*Clonophis*—Kirtland's snake.
   Genus—*Opheodrys*—green snake.
C. Dorsal scales not keeled.
   Genus—*Liopeltis*—grass snake.
   Genus—*Diadophis*—ring-necked snake.
   Genus—*Bascanion*—black snake.
   C[1]. Anal plate entire. Dorsal scales all or part of them keeled.
   Genus—*Pituophis*—pine snake.
   Genus—*Thamnophis*—garter-snake (viviparous).
   C[2]. Dorsal scales not keeled.
   Genus—*Lampropeltis*—chain snake, milk snake.
   Genus—*Osceola*—scarlet snake.
   Genus—*Cemophora*.
D. Rostral plate recurved and keeled. Anal plate divided. Dorsal scales keeled.
   Genus—*Heterodon*—hog-nosed snake, spreading adder.

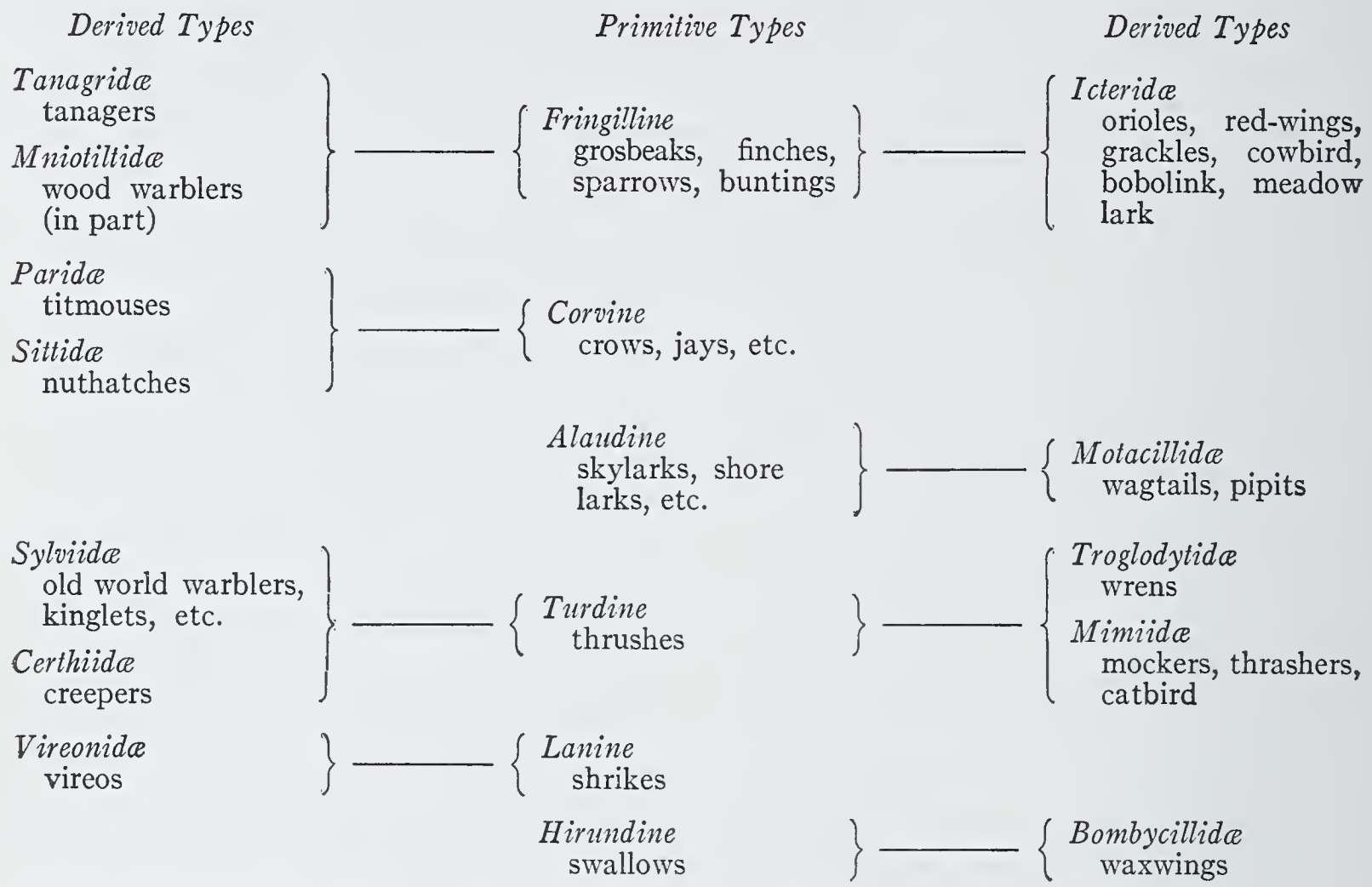
group (the genera *Setophaga* and *Wilsonia*), and possibly *Mniotilta*, *Helinia*, and *Protonotaria* are outliers. Interestingly, too, the Chat, Black and White Creeper, Swainson's, the Prothonotary, and to add another, the Worm-eating Warbler, constitute in the main monotypic genera. In *Icteridæ* the monotypic Bobolink, the Cowbird, and the Meadow Larks are outliers compared with the Orioles and their neotropical allies, with the Grackles and the Red-wings, which

are central—*i. e.*, embody more closely our idea of Icterine characteristics, though the Grackles, as Coues has said, grade toward the *Corvidæ*. In other words, the *Icteridæ* include types that are far from presenting that solidarity which we expect in a family group. It represents, however, the best that we can do under the circumstances of our present state of taxonomy. I think it was an eminent British naturalist, the late Professor Flower, who once defined a system of classification as a row of pegs to hang ideas on. We must change the pegs as we gain new light through investigation and so hang our ideas on a different arrangement. The pegs are movable, but we are not yet in a position to move them. One's idea concerning the relationship and arrangement of the families of birds is largely a matter of the printed page—most of us know our taxonomic succession from such books as Coues and Chapman. Those of us who are visualizers see, in our mind's eye, the family name as it exists in print, with its descriptive text and the succession of genera and included species. One family follows another and we have very clear notions of the relative positions of these family groups between the covers of a book. But does this really represent the facts? In the nature of things, so far as books go, any other arrangement would seem quite illogical. It is undoubtedly the best that we can do. Criticism of the existing scheme is much like the rude sign that was nailed over the organ in the church of a western mining town of the old days. It read, "Please don't shoot at the choir, it's doin' the best it knows how." At one time the Thrushes headed the procession, now the Loons greet us on the opening page. No linear arrangement, however, can be entirely satisfactory. Volumes like Ridgway's great work on "The Birds of North and Middle America" deal with related groups in more or less close juxtaposition, each group of related families considered on its own merits and without reference to any stem to stern scheme. In a work of a single volume this would be quite impossible, the families have to be arranged in some sort of fashion and the current arrangement is admirable as far as it goes.

The scheme I have in mind merely interests me in trying to solve certain relationships. Among the North American Oscine families which represent well-defined groups in the general chaotic mass of forms, unquestionably the Thrushes, the Finches, the Swallows, the Crows and Jays, the Larks, and possibly the Shrikes are clearly defined assemblages. On the other hand, the Wrens, the Mockers, the Pipits, the Waxwings, the Vireos, the Tanagers, the Wood Warblers, the Sylvias, the Icteridæ, the Nuthatches, Titmouses and Creepers

are not so well defined; they have very irregular frontiers that tend to fuse with one another and with the more central and better defined groups.

On the suggestion that a system of classification is a row of pegs to hang ideas on, I have sketched out a chart to indicate the possible relations of certain Oscine families:



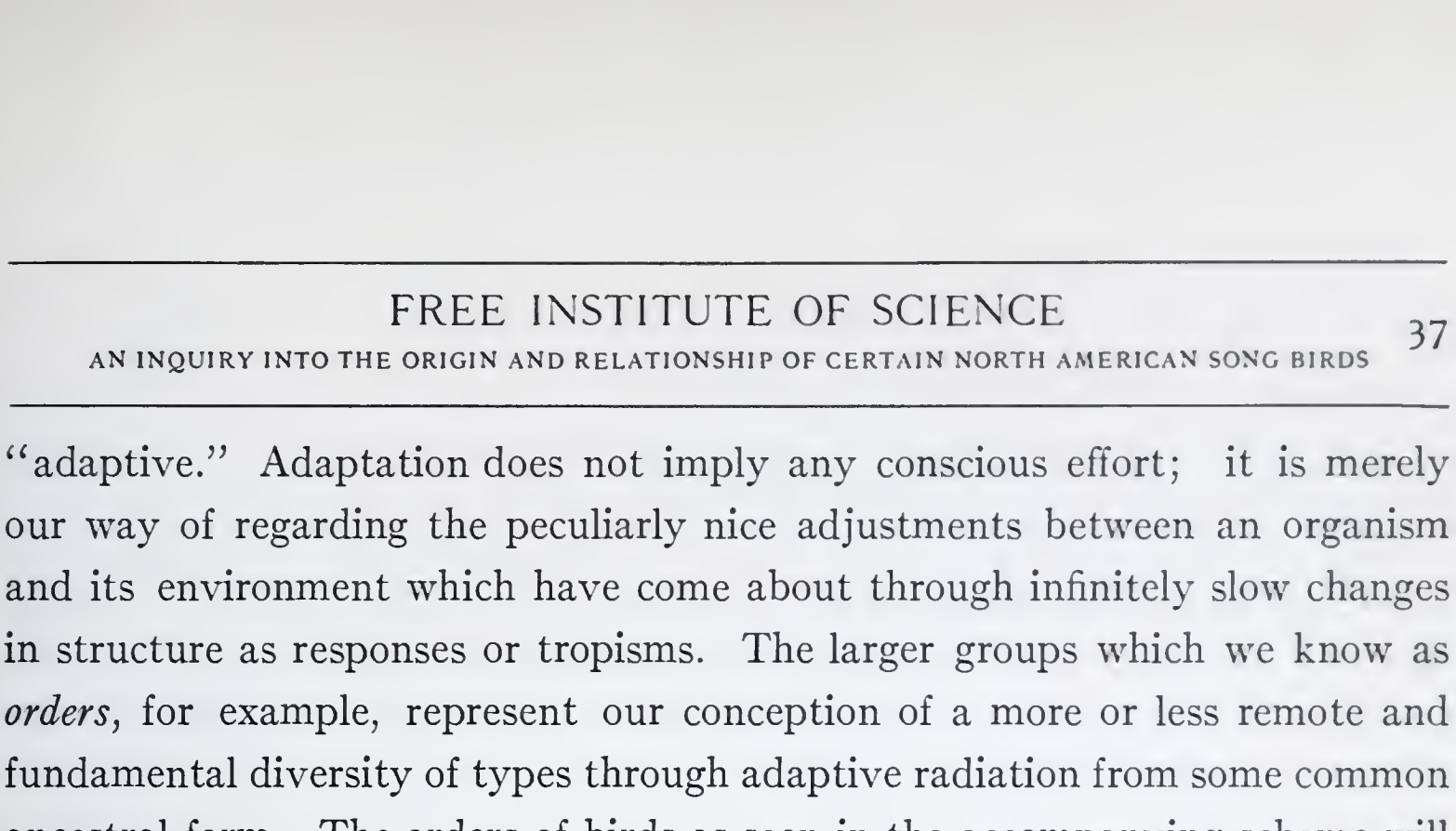
TENTATIVE SCHEME OF FAMILY RELATIONSHIPS AMONG NORTH AMERICAN OSCINE PASSERINE BIRDS. The six central groups are regarded as representing the more primitive types which have given rise to the derived types through adaptive radiation.

2

In seeking the origin of our bird fauna two points of view present themselves—one *geographical*, the other *structural*. Both of these, however, must be considered together as intimately related factors which have brought about the great variety of existing forms. The term "geographical" is here used in somewhat different sense from that with which it is commonly associated in our minds. It refers, in this sense, not so much to wide areas of the earth's surface as to certain environing influences, which are present in these areas as *situations* to which living beings have become variously moulded through "adaptive radiation."* "Radiation" implies a spreading out of individuals from some more or less remote parent stock into diverse situations, the particular conditions of each situation being met by changes in structure which are

* Osborn, H. F.: *The Age of Mammals*, New York, 1910, 22–30.

"adaptive." Adaptation does not imply any conscious effort; it is merely our way of regarding the peculiarly nice adjustments between an organism and its environment which have come about through infinitely slow changes in structure as responses or tropisms. The larger groups which we know as *orders*, for example, represent our conception of a more or less remote and fundamental diversity of types through adaptive radiation from some common ancestral form. The orders of birds as seen in the accompanying scheme will illustrate this point. Each represents a splitting-up among the descendants of some primitive winged creature to fill the various situations presented by the diversity of environment—the various aquatic types, the primitive rapacious group, the purely ground-loving forms, like the fowls and pheasants, the common plover, gull and pigeon group and the several arboreal orders. Each of these has again split up through many minor adjustments which represent matters of detail to the systematic ornithologist in the arrangement of families, genera, species and geographical races or sub-species.



SCHEME OF THE ORDERS AND SUB-ORDERS OF BIRDS (ADAPTIVE RADIATION).
From the author's "Synoptical Review of the Animal Phyla."

The underlying problems in the life of every individual animal are resolved into the nature of its food and the means of securing it and into the production and care of offspring. These present the entire panorama of existence. Securing of food means various ways and means of locomotion as conditioned by the

peculiar nature of the environment in which the food is found and the development of *habits* in the effort to secure it. Intimately related to this is the problem of rearing young and methods of escape from enemies. Many different kinds of animals dwell in the same kind of environment, forming a *community* in which the different species have struck a certain balance in the struggle for existence. Animals have by no means all reached a perfect adaptation in relation to their surroundings. What we observe is a more or less perfect or imperfect approach to this. No species is probably in absolute perfect adjustment. What we term a "rare species" is one which is far from having attained to a perfection in adjustment in comparison with abundant and widely spread forms. The rare species may represent one that is on the way to attainment or one that is dropping down in the struggle for place.

In such a highly specialized group of birds as the Passeres, in which the numerous forms are separated from one another by comparatively slight external features, the question of relationship among the various families is often, as we have seen, extremely vague. Only once do we find a cleavage in the group that has any deep morphological significance—the structural difference in the syrinx or vocal organ that separates a distinct clamatorial or non-melodious group from an acromyodian or oscine group—the so-called "song birds"—in which the intrinsic syringeal muscles have reached a more or less perfect development. Elsewhere, as we have seen, it is a question of purely *external* differentiation in modifications of wing structure (reduction of primaries), of the form of the bill, of peculiarities of the tarsal envelope and of differences in the manner of moulting—all of them structures of epidermal origin save that of the beak alone, in which the mandibular and maxillary elements have been more or less modified in relation to the nature of the food. Stripped of its feathers and the outer sheathing of its bill, any passerine bird, except for the matter of size, looks about like any other one—a striking contrast to the more clearly defined and structurally deeper distinctions in any order of mammals and reptiles. This is more or less true of the entire class of birds. Geologically speaking the passerine bird is a comparatively recent production, an offshoot from the primitive avian stock through the influences of adaptive radiation, and there has hardly been time enough to effect any very deep structural changes among the members of its type.

Unlike the paleontological record among reptiles, mammals, fishes and amphibians, the bird line has left few fossil remains, and in our efforts to con-

struct a scheme of phylogenetic relationships among modern birds we are forced to frame altogether tentative hypotheses on the small margin of existing likenesses. At the outset we are met with a host of superficial resemblances and differences that make "confusion worse confounded." How many points of similarity among certain groups of birds may be purely analogous likenesses, the result of similar conditions of life (convergent evolution) and not in any way genetic? And yet how are we to know whether they mean the one thing or the other? It is fairly clear, however, that the breaking up of the passerine type into *families* is a comparatively recent event as a result of variations in the manner of life and the nature of the food. What the relationships of these families are among themselves can only be surmised, and resemblances, as I have said, may be misleading. There appears to be a critical point of evolution in the number of the primary wing feathers and possibly also in the lamination, fusion or entire scutellation of the tarsal envelope. What these superficial features signify is, in our present state of knowledge, an unknown factor. The bill has undoubtedly been modified in relation to food and it is reasonable to believe that it is an advance in wing specialization where we see families in which certain species have the first feather of the ten primaries shortened, as in certain Thrushes and Vireos, a fact that points to the probable method of reduction in the forms with nine primaries.

3

The causes that underlie specific differentiation, and in more remote time those deeper tissue changes which we now recognize as generic and family distinctions, may quite possibly be traced to influences arising from certain internal glandular structures, the activities of which produce substances of a physico-chemical nature known as *hormones*, that are discharged into the blood and lymph. In the development of biological inquiry, particularly along the line of inherited characters, there has been revealed, through the researches of Mendel and later investigators who have followed in his steps, a definitely recognized *plan of heredity*. It has been further shown that the transmission of characters is accomplished through the chromosomes, and the question arises as to what is the basic physico-chemical nature of this transmission. In the particular field now under review—the differentiation of type among passerine birds—one is led to suspect that the differences observed may have originated in sex cells which have been impressed by hormones from the body at large,

and that the somatic or bodily qualities, though adhering to the general type, are subject to a slow process of change through the generations, becoming gradually modified by environing influences along lines of adaptive radiation.

The differential characters of a group of species in the same genus are of the most superficial kind, as we have seen, affecting mainly epidermal structures, notably in the pattern and quality of color. Among the species of a genus, however, there appear variations of a deeper nature—nervous reactions as exhibited in differences in habits, song, migratory range and so forth. Take the case of a genus of birds like *Dendroica* among the wood warblers, for example. As far as plumage is concerned, it is among the males during the breeding season that marked differences exist and this is true also in regard to the expression of the sex impulse in song. There can be little doubt that this outburst of song and of more or less vivid color pattern is a by-product of sex gland activity fundamentally related to the production of hormones by certain cells of the glands. Each group of individuals that constitute a species have the same kind of hormones and different from those of a closely related species. The shade and pattern of color and the specific peculiarity of vocal expression have remained quite constant over a long period of time through the perpetuation of the same kind of hormone or physico-chemical secretion in succeeding generations. The establishment of specific characters, their *fixedness*, in other words, is the result of continuous segregation of the mating individuals which possess the same type of hormone. A departure from this results in hybridism, as seen in the case of the two species of *Helminthophaga* where the Blue-winged and Golden-winged Warblers (*H. pinus* and *H. chrysoptera*) have crossed and produced the Lawrence and Brewster types of Warbler, a very clear illustration of Mendel's law.

The more or less close relationship of certain species within the genus *Dendroica* is seen in the similarity of the juvenal plumage, a fact which indicates a comparatively recent break-up of the original type into several species (the Black-poll and Bay-breasted Warblers), due most probably to slight changes in the nature of the hormones induced by some factor of environment.

What is true of the birds just considered is true of all other forms. We draw the logical conclusion also that at a more remote time a common *family* type broke up into its several genera as we now see them, and still more remotely a generalized ancestor, representing what we now call an *order*, split up into forms which represent our conception of the common family type. The

more primitive variations, those which define the larger groups like orders, affect deeper and more fundamental structures, but all alike, superficial as well as deep, have been perpetuated by inconceivably slow changes affecting the hormone constituents of the organism in their interaction through the somatic and germinal tissues. Whether variations in the hormone secretions of an organism may arise spontaneously without the evident influence of environment, as in the case of rapid mutations, and thus produce new species, is beyond the scope of the present paper. The researches of DeVries and other students of heredity indicate that this method may occur under certain conditions.

I am aware that this subject is somewhat vague and speculative. To most of us the concrete bird is the vitally interesting problem—the life histories and distribution of species, that is the thing that holds our interest, and very rightly so. However, these questions of relationship also are vital problems, full of interest and very fruitful in getting at some fundamental facts concerning the genealogy of modern birds and likewise in showing the difficulties that beset the path of the systematic ornithologist.

THE THREE-ELECTRODE BULB AS DETECTOR AND AMPLIFIER OF RADIO SIGNALS

LESLIE BIRCHARD SEELY

IT IS not proposed in this treatment of the three-electrode bulb to discuss in any degree the various tuning devices used in radio receiving circuits, nor to discuss the relative merits of the various circuits in use at the present time. Advance is so rapid along these lines that any such treatment would be antiquated before it left the press. It is proposed, however, to state, as simply as may be, the fundamental physical properties of these tubes, and to indicate in a general way their use in the detection and amplification of radio waves. Any digression from this immediate discussion will be for the purpose of clarifying the phenomena pertaining to the object as stated.

The use of three-electrode bulbs as rectifiers of alternating currents, as senders, receivers, and amplifiers of radio signals, has been made possible by the discovery of the Edison effect and the development of our knowledge of the electron. The studies along the immediate line of development of the bulbs themselves by such men as Elster, Geitel, Fleming, Richardson and Langmuir, to mention only a few of the host of contributors to the subject, have been indispensable. The invention of the three-electrode bulb itself must be accredited to De Forest.

THE EDISON EFFECT

The Edison effect may be demonstrated as follows: A metal foil is placed over a portion of an ordinary carbon electric light bulb which is attached to a direct current lighting circuit. A battery and galvanometer are connected in series between the foil and the negative end of the filament. After the lamp has been heated so that the glass has become sufficiently conducting a current will pass through the galvanometer provided the battery is connected so that the current flows through the tube from the foil to the filament. If the battery is reversed, no current will flow. When connected as shown in Figure 1 the galvanometer needle will be deflected, but if the battery is reversed it will not be deflected. It has been shown that the current in the battery circuit passes

through the glass and the space between the glass and the filament as a stream of electrons flowing in the opposite direction, *i. e.*, from the filament to the foil.

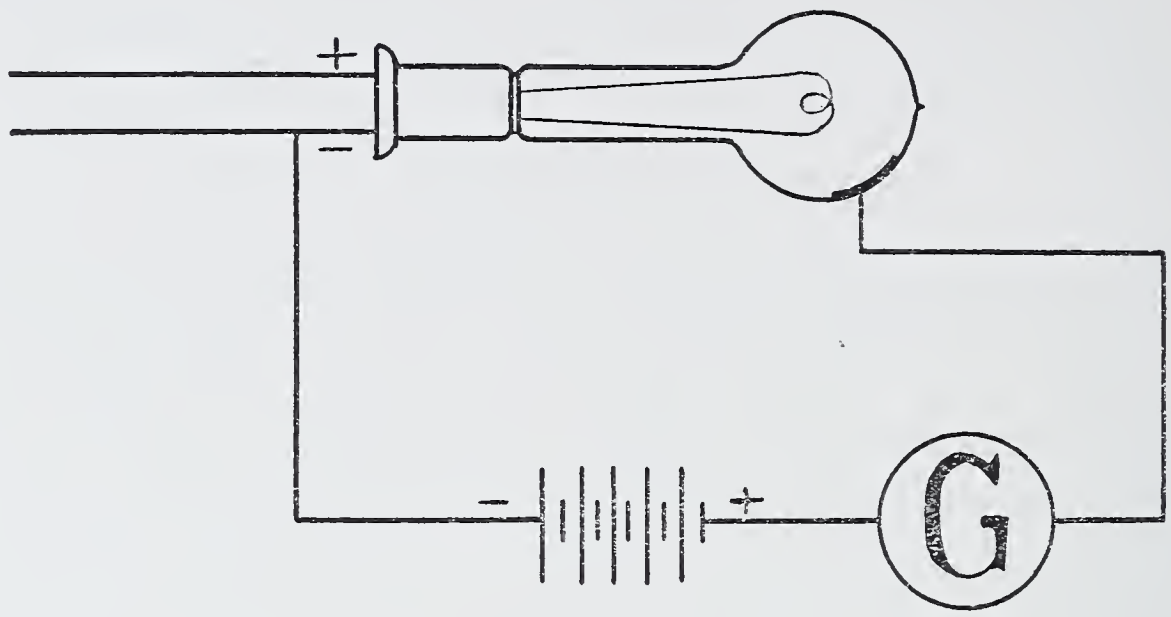


Fig. 1.

If now the battery be replaced by a source of alternating current of suitable voltage, the galvanometer detects a direct pulsating current in the direction indicated above.

EMISSION OF ELECTRONS FROM HOT METALS

The phenomena described above will be shown to depend directly upon the emission of electrons from the filament when it is heated; or, to state it generally, upon the thermionic emission of electrons from metals.

When any pure metal is heated, electrons emerge from its surface with all possible speeds between zero and, possibly, that of light. The average speed, however, is proportional to the absolute temperature of the metal. The number per second of electrons emitted from a unit of surface of the metal also varies with the temperature, being proportional to $T^{\frac{3}{2}}$. The entire phenomenon so closely resembles the evaporation of molecules from the surface of a body of liquid that for most purposes the fundamental conceptions and indeed the mathematical relations involved in evaporation may be considered as holding for the thermionic emission of electrons.

The energy of the electrons within the metal is determined by the absolute temperature, T. In order to escape from the surface of the metal, a portion of this energy must be expended. The amount of work thus done by each electron

is characteristic of the metal and is called its work function. It will be noted that this work function is entirely analogous to the heat of evaporation in liquids. The amount of emission per sq. cm. of surface expressed in terms of current intensity has been determined by Richardson to be expressed by the equation

$$i = AT^2E - \frac{e\phi}{KT} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where A is a constant which according to Dushman is the same for all metals, K is the Boltzman gas constant from the equation $pv = ukt$, T is the absolute temperature, and $e\phi$ is the work function referred to above.

THE ELECTRONIC ATMOSPHERE

From what has been stated above it will be seen that to acquire a maximum emission of electrons a metal must be chosen which has a low work function together with a melting point which will permit of its use at very high temperatures. Such a combination of qualities has been produced by Langmuir in a tungsten filament having a surface coating of thorium. All metals, at least when greatly heated, may be considered as being surrounded by an atmosphere of free electrons. The density and extent of this atmosphere will depend primarily upon the temperature of the metal and the magnitude of the work necessary for an electron to perform at the surface of the metal in making its escape.

If conditions surrounding the metal be such that the electrons are removed from the immediate neighborhood of the metal as rapidly as they emerge, a constant flow at a fixed rate will result as determined by equation (1).

If, however, the conditions surrounding the metal are such that an atmosphere of electrons or of negative gas ions is formed then the rate of emission is in effect modified by the return of some of the electrons into the metal under the action of the forces of electrical attraction and repulsion. The close resemblance of this phenomenon to that of liquid evaporation is apparent.

THE TWO-ELECTRODE TUBE

If now in place of the foil on the bulb in Figure 1 we place an electrode inside the bulb and connect it with the positive pole of the battery while the negative end of the filament be connected with the negative pole of the battery,

the electrons escaping from the heated filament will pass in a stream from the heated filament to the cooler electrode. This is in effect a current passing in the opposite direction. If the battery be reversed no current will pass. This bulb will then serve as a "valve" or rectifier of alternating currents. If the field created by the anode be strong enough so that all of the electrons escaping from the filament pass over immediately the current will assume a value consistent with the rate of electronic emission. If the field be weak, *i. e.*, if the potential of the anode be not sufficiently high, not all of the escaped electrons will pass over to the anode since some will be driven back to the filament because of the space charge created by those electrons already emitted. Below a certain maximum current strength fixed by the temperature and nature of the filament or cathode, the amount of current passing will depend on the potential of the anode as compared with the negative end of the filament. This maximum value is the intensity of current indicated by equation (1). Below this value the current is proportional to the square root of the cube of the applied voltage. Langmuir has shown that for values of i below that given by equation (1),

$$i = 2.33(10)^{-6} d^{-2} V^{\frac{3}{2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2),$$

where the electrodes are parallel plates at a distance d from each other. The form of the equation will vary with the shape and relation of the electrodes, being

$$i = 14.65(10)^{-6} r^{-1} V^{\frac{3}{2}}$$

where the filament is a straight wire and surrounded by a coaxial cylindrical plate of radius r.

When the potential of the plate or anode is increased so that the resultant field becomes neutral at the surface of the filament the maximum current will have been obtained, for the electrons will then pass from the filament to the plate unimpeded by any space charge. In reality this neutral zone may be a considerable distance beyond the surface of the filament, for it is to be supposed that the electrons having escaped from the filament will in the average case be carried some distance beyond its surface by their own velocities.

Many of the above facts may be shown as in Figure 2. The heavy curve gives the current plotted against the temperature of a given cathode as determined by equation (1). The broken curves give actual values for the current intensities obtained by using three voltages $V_1, V_2,$ and $V_3,$ chosen so that they

are less than required to neutralize the effect of the space charge, so that the entire emission is not represented in the current obtained.

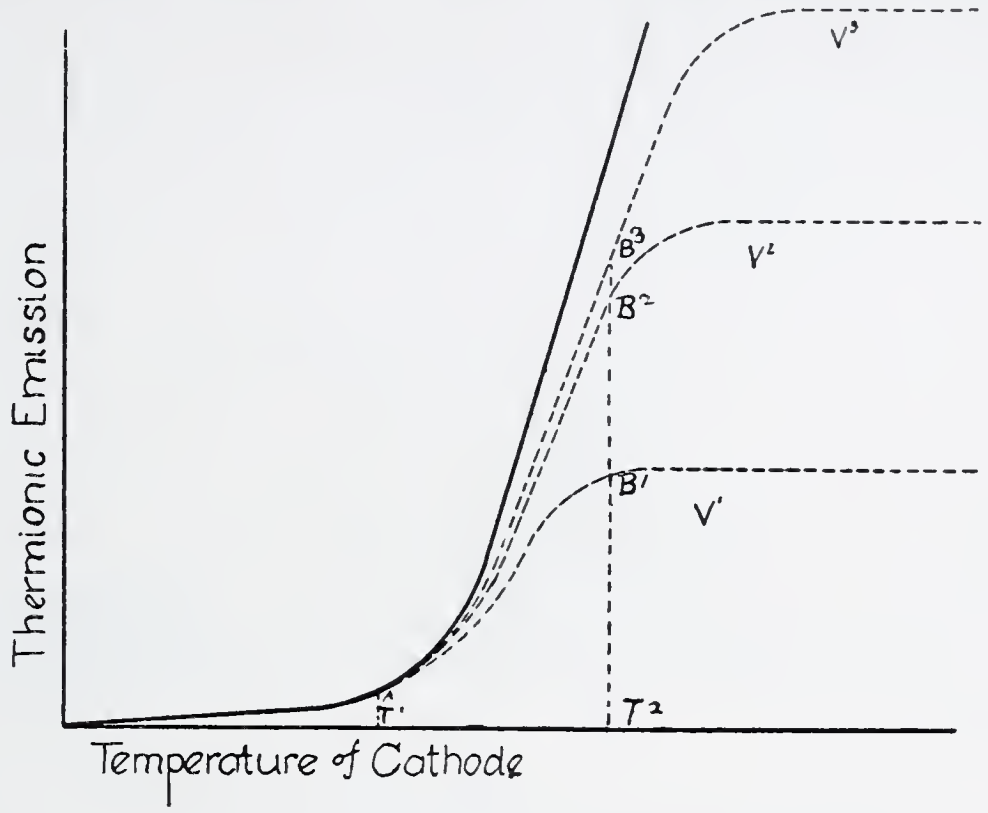


Fig 2.

THE CHARACTERISTIC CURVE OF THE TUBE

It is clear from the curves in Figure 2 that for values of V below that necessary to utilize the entire emission at a given temperature any change in the impressed voltage will produce a change in the current; but the ratio in the increase of the current to that of the voltage is not constant. Suppose, for instance, that at the temperature T_1 of the filament, and at the voltage of V of the plate, the tube will be working on the knee of the curve. The plate current will be represented by T_1A. Now any increase in the voltage will not produce a corresponding increase in the current, for the entire emission is already being utilized since at A the curve for V_1 lies on the curve of the emission. When, however, the temperature of the filament has been increased to T_2, the voltage V_1 will not utilize the entire emission. The current will have risen, however, to a value of T_2B_1. If now at this new temperature T_2 a higher voltage V_2 be used the value of the current will rise to T_2B_2, and for voltage V_3 it will be T_2B_3. None of these current values will equal that intensity indicated by the curve of emission although T_2B_3 will closely approximate it. In other words, the voltage of the plate in none of these instances is sufficient to overcome the effect of the space charge.

Consider now what occurs when a fixed voltage such as V_1 is applied to the plate and the temperature of the filament is varied. With a temperature such as T_1 the current will be represented by T_1A, by increasing the temperature to T_2 the current may be made to assume a value represented by T_2B. Thereafter any increase in the temperature will produce no further increase in the current since the curve becomes approximately parallel with the temperature axis.

The above consideration shows that the amount of current passing through a tube depends first, on the temperature of the filament or rather on the rate of emission as determined by equation (1) and second on the voltage of the plate as compared with the filament. Also, that for current values below that indicated by equation 1 the current value is fixed by the plate voltage and is determined by equation 2. It is true also that for increasing voltages the values gotten as indicated by equation 2 approach the value indicated by equation 1 as a limiting value.

THE THREE-ELECTRODE TUBE

It occurred to De Forest that the space charge and thus the plate current might be controlled by a third electrode lying between the plate and the filament. This electrode made in the form of a lattice or grid so as to allow the passage of the electrons has become the means by which the tube has revolutionized the detection and amplification of radio signals.

In the usual form of the tube the filament occupies a central position, and is surrounded by a cylindrical grid or lattice; and this in turn is surrounded by a plate, also cylindrical in form. All of the electrodes are placed coaxially. For clarity the tube is usually represented in diagram as in Figure 3.

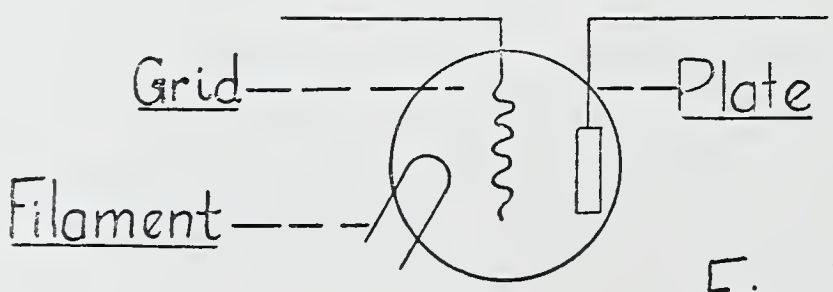


Fig. 3.

In order to understand the working principle of the device consider Figure 4. The "A" battery is a battery or other source of current for controlling the temperature of the filament. The "B" battery is for controlling the potential of the plate as compared with the negative end of the filament. The grid is

connected to aerial or other source of variable potential. If now the temperature of the filament and the potential of the plate be so regulated that the tube

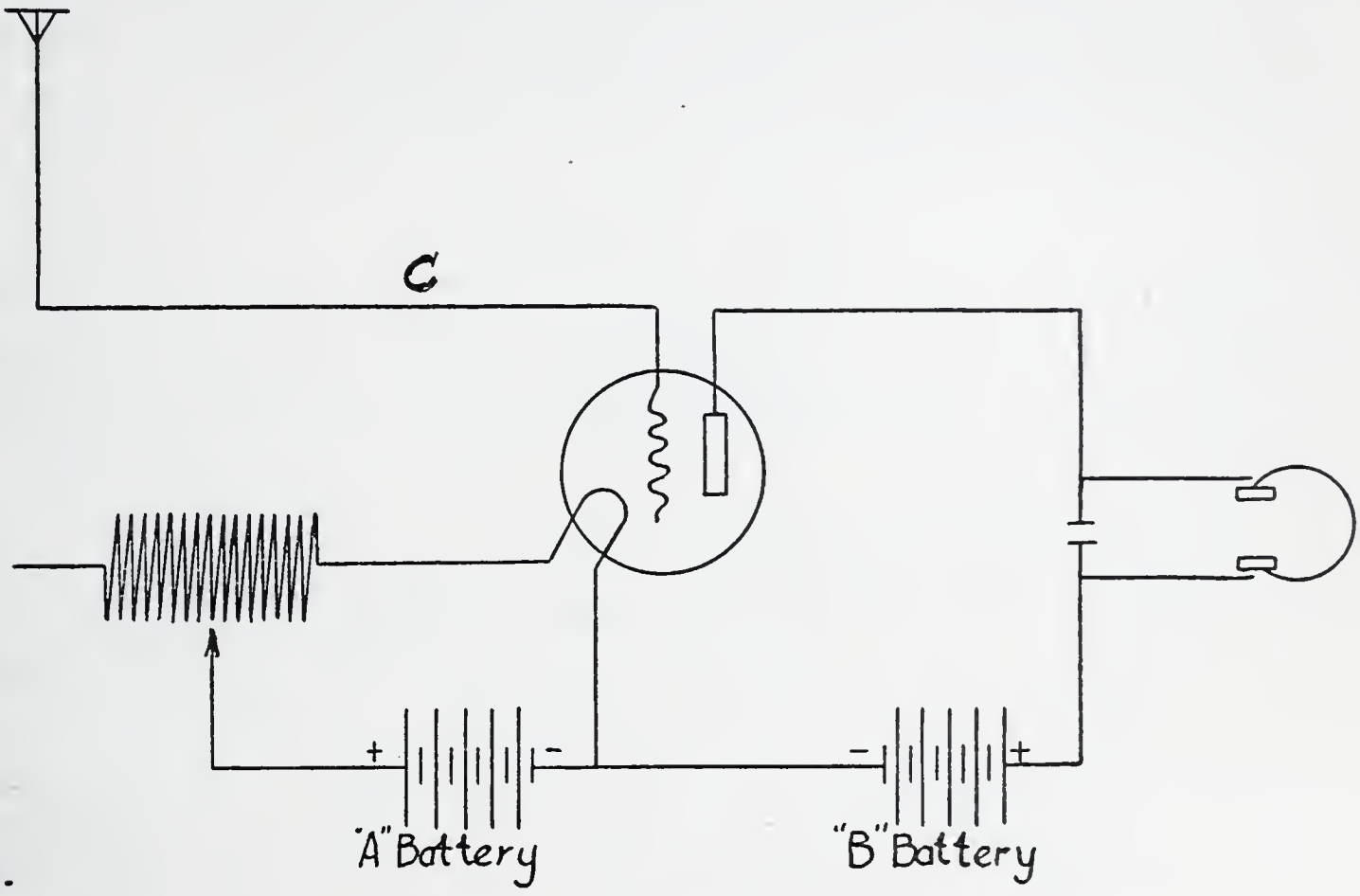


Fig. 4.

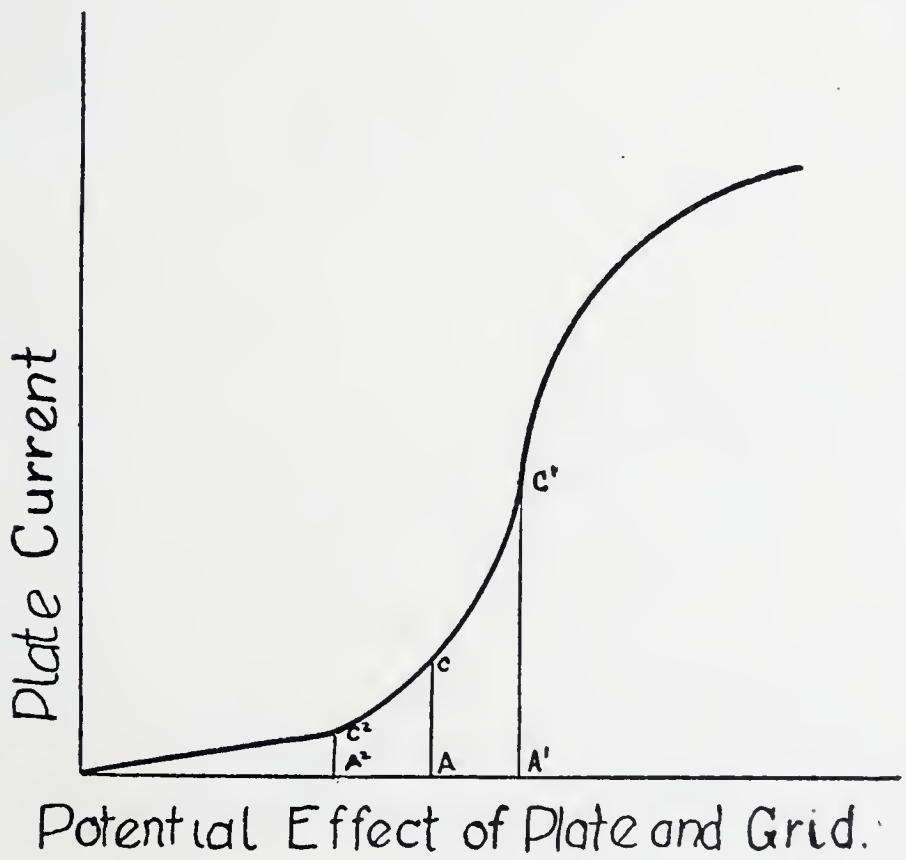


Fig 5.

is working on a knee of its characteristic curve it becomes extremely sensitive to changes of potential in the grid. This is merely stating that small differ-

ences of potential on the grid are very effective in varying the effect of the space charge in the tube. So a small increase on the potential of the grid may result in a relatively large increase in the plate current. This may be seen more readily in Figure 5, where the characteristic current curve of the tube is gotten by plotting it against the potential of the grid.

RADIO FREQUENCY

It is not the purpose of this paper to discuss the use of the three-electrode tube for broadcasting radio signals, but in order to understand the action of a receiving tube we must know something of the nature of the energy which it detects and amplifies.

All tube distributing stations operate on a continuous wave, usually spoken of as "CW." Under certain prescribed conditions a sending tube may be made to oscillate. If such a tube be connected to an aerial, it will alternately charge the aerial positively and negatively in such a way as to cause it to radiate electro-magnetic waves. In effect the tube is distributing through the aerial energy from a local circuit in the form of electro-magnetic or Hertzian waves. For a given tube and circuit the frequency of these waves is constant, but their intensity may be greatly varied by the action of sound waves on a microphone in the local circuit. This continuous wave then is a train of waves consisting of alternate high and low potentials spreading out in all directions from the broadcasting aerial. They travel with about the speed of light. The wave length of such a train is constant and is determined by the distance between the like phases of any two succeeding waves. Thus a 400-meter wave length indicates that a distance of 400 meters or about ¼ mile intervenes between any "high" and the next succeeding "high" or between any "low" and the next succeeding "low." These alternate highs and lows pass any given point at about the speed of light, so that about 750,000 complete waves pass the given point per second. A receiving aerial lying in such a wave train will have its potential raised and lowered as each succeeding wave passes. This produces a high frequency oscillating current in a wire connecting the aerial with the earth. Likewise, if such an aerial be connected with the grid of a three-electrode tube it will cause rapid alternations in the potential of the grid. If the temperature of the filament and the potential of the plate of such a receiving tube be so regulated that the tube is passing a current represented by a point on the lower "knee" of its characteristic curve (see

C_2, Figure 5), then each small increase in potential of the grid will result in a relatively large increase in the plate current while the high potential phase of the wave is passing. The intervening low potentials, however, will produce but little effect on the plate current. A little study of Figure 5 will make this clear. Suppose the plate with potential A produces a current indicated by AC. This is with the grid neutral. Then suppose the grid has its potential raised so that the resultant field in the tube is the same as that produced by a plate potential A_1. A current represented by A_1C_1 will result. Corresponding negative potential on the grid, however, such as would result in a field like that produced by a plate potential A_2, would reduce the value of the current to that represented by A_2C_2.

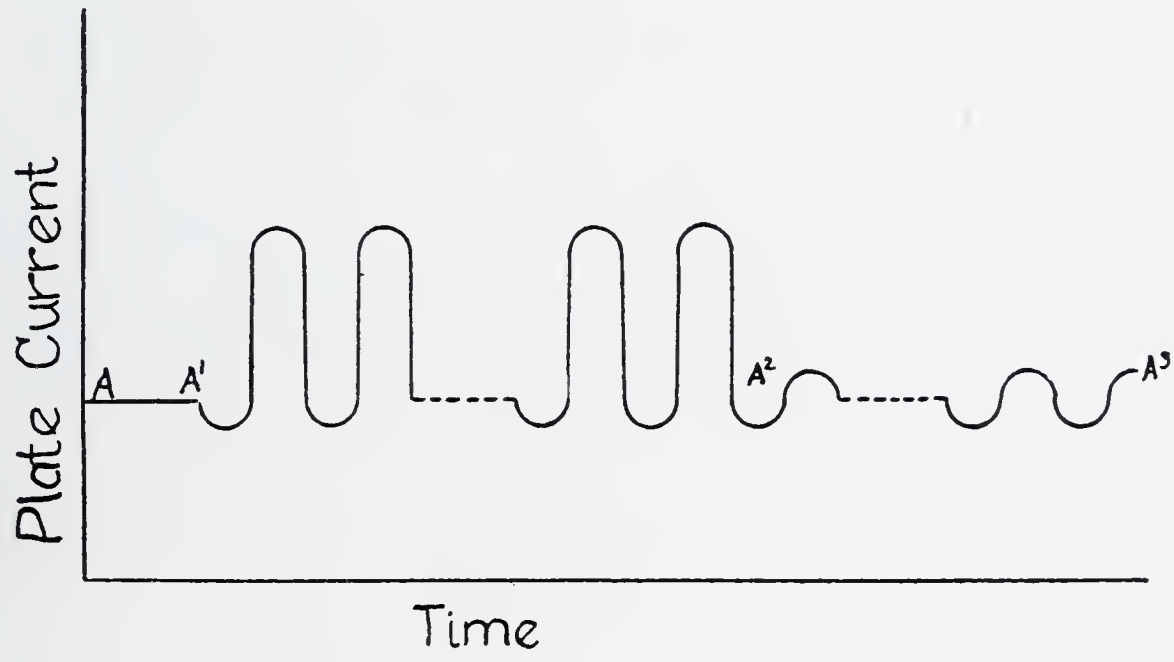


Fig. 6.

These conditions may be somewhat clarified by plotting actual plate current values against time, as shown in Figure 6. Let the current of the tube with grid neutral be represented by the line AA_1. Now if the 400-meter continuous wave from a sending station be considered as acting on the aerial and so on the grid of the receiving tube, the plate current would become a series of pulses something like those represented in A_1A_2 or A_2A_3, each of these pulses would persist about $\frac{1}{750,000}$ of a second. The intensity of each pulse would depend upon the intensity of the wave passing the aerial. Suppose they should assume some value such as represented in A_1A_2 and retain that intensity for .002 sec., then drop off as represented in A_2A_3 for an equal period of time. A_1A_2 would then consist of about 1,500 intense pulses and A_2A_3 would consist of the same number of relatively faint pulses.

Now the individual pulses in A_1A_2 or A_2A_3 are of entirely too short duration to affect a telephone receiver connected in the plate circuit (see Figure 4), but the change in intensity of the pulses occurring at A_2 would produce a definite click in the telephone. If at A_3, the intensities again changed to values like those represented in A_1A_2, another click would be heard. In other words, the individual waves in A_1A_2 or A_2A_3 would not be detected by the telephones but the changes in intensity at A_1A_2, and if the conditions were repeated, at each successive point in time represented by A_1, A_2 or A_3 would be detected. This click would occur at intervals, under the conditions set forth, of about .002 sec. The result would be a musical tone in the telephones. This tone would be a reproduction of the tone affecting the microphone in the sending station. Now the individual pulses are said to occur at radio frequency while the changes in intensity of these pulses occur at audio frequency.

CHANGE FROM RADIO TO AUDIO FREQUENCY

Suppose that during the interval of time represented by A_1A_2 in Figure 6, the average current be represented by A_1A_2 in Figure 7, while during the time

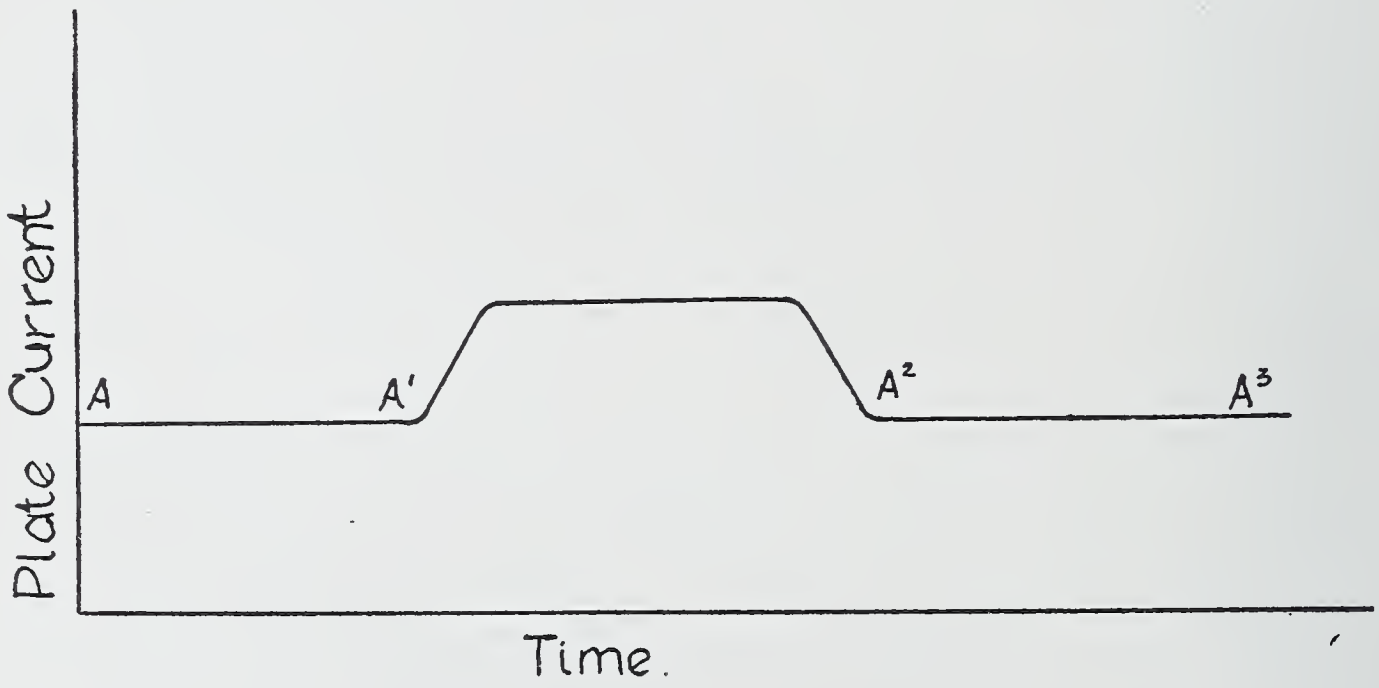


Fig 7.

interval represented by A_2A_3 in Figure 6 the average current be represented by A_2A_3 in Figure 7, then $A_1A_2A_3$ will represent a current wave of audio frequency, and it is therefore able to be detected by the telephones. This same effect may be produced by introducing a condenser and grid leak in series with the aerial and grid as at C in Figure 4. The simple grid and leak properly ad-

justed* act as a reservoir into which the single pulses of radio frequency are poured. In this case the condenser is filled during intense pulses as in A_1A_2 and emptied during the period of faint pulses as in A_2A_3. Thus the variation of the plate circuit is actually at audio frequency. With the simple circuit it will be understood that the actual pulses at radio frequency appear in the plate circuit while with the grid and leak the variations in the plate circuit correspond to the variations in intensity of the radio pulses, and are therefore at audio frequency. Since the variations in intensity appear as a result of the action of sound waves on a microphone in the sending circuit, the audio frequencies in the receiving circuit are electric currents which in a telephone will reproduce the sounds which caused the variations at the sending station.

AMPLIFICATION

The electron stream from filament to plate is so flexible an instrument that it will under proper adjustment of circuits reproduce in the plate current either the radio frequencies or the variations in intensity which are at audio frequency. By carrying the output of the first tube to the grid of a second tube, etc., as many steps of amplification as are desired may be had. It has been pointed out that small changes in potential of the grid may produce relatively larger changes in the intensity of the plate current. From a study of the characteristic curve of the tube in Figure 5 this will be seen to be especially true when the tube is working on the knee of the curve. But while it is true that more magnification may be had under these conditions, there is also more distortion in the relation of the increases and decreases in intensity. So for most satisfactory amplification of sounds the approximately straight part of the characteristic curve must be used. See C, Figure 5. Here the increases in the plate current are approximately proportional to variations in the grid potential.

For the detection of code signals where distortion is no disadvantage the knee of the curve is more satisfactory, because of the much greater magnification which may be had.

In actual three or four step amplification it is customary to carry the radio frequency through one or two steps and then amplify further at audio frequency.

* The grid side of the condenser must be kept negative with respect to the filament. In some types of work this is insured by the use of a grid battery.

SUMMARY

At high temperatures all pure metals emit electrons. If two electrodes unequally heated are introduced into a vacuum tube the electrons will pass from the hot to the cold electrode, provided the potential of the cold electrode is kept high at a relatively higher potential. If the cold electrode or plate is not kept sufficiently high to insure the immediate transfer of all emitted electrons, a space charge is formed which rapidly reduces the amount of plate current. A grid or lattice introduced between the two electrodes gives a very efficient control of the space charge and therefore of the plate current. If this grid be connected with an aerial any differences of potential of the aerial will, through the grid, be reproduced in magnified form in the plate current.

Hertzian waves at radio frequency may have their intensities varied by the action of sound waves on a microphone in the sending circuit. These waves with their variations may be reproduced in the plate circuit of a receiving tube, and so the sounds affecting the sending circuit may be reproduced.

These reproduced waves in the receiving circuit may be amplified by sending the plate current into the grid of a second tube, etc., for as many steps of amplification as are desired.

For the detection of signals the tube is most efficient when working on a knee of the curve. Where distortion of relative intensities is undesirable, the relatively straight portion of the characteristic curve lying between the knees should be used.

THE DETECTION OF METHANOL IN THE PRESENCE OF ETHANOL

CHARLES HERBERT LAWALL

THE detection of methanol in the presence of ethanol has assumed more than academic importance recently on account of the frequent employment of denatured alcohol in liquors intended for medicinal or beverage purposes.

The identification of methanol when it is the only member of the alcohol group in solution presents no great problem, but when it constitutes but a small proportion of the total alcoholic content peculiar difficulties are encountered which complicate the situation and make it necessary to pursue indirect methods that are always less satisfactory than direct positive identifying tests.

The use of the immersion refractometer in conjunction with the specific gravity is a physical method of recognized value, indirect though it be, and this method is adopted as one of the alternative official methods of the Association of Official Agricultural Chemists, it being originally proposed by Leach & Lythgoe (*J. Amer. Chem. Soc.*, 1905, **27,** 964).

All of the chemical methods that have thus far been proposed have been based upon the production of derivatives or decomposition products of the methyl and ethyl groups respectively, and the identification or differentiation of these resulting products by appropriate tests.

One of the simplest and least satisfactory of the older methods, which is frequently quoted in literature of twenty or more years ago, and for which no original reference could be found, is the conversion of the radicles into the respective salicylates. It is true that the odor of methyl salicylate is distinctively different from that of ethyl salicylate, when they are observed separately, but in the presence of a preponderating amount of the latter ester the odor of the former is obscured, and there being no other test than odor for distinguishing them in a mixture, the method is unsatisfactory and unscientific.

One of the earliest of suggested methods is that of Riche and Bardy (*Compt. Rend.*, 1875, **80,** 1076). This, which is exceedingly tedious and time-consuming, depends upon the formation of methylanilin violet. It is one of the official methods of the Association of Official Agricultural Chemists and

is of advantage as a confirmation test for medico-legal cases, for the colored solution or dyed strips of wool may be kept indefinitely and exhibited as evidence. The test is in no sense quantitative, but is one of the most delicate that has been proposed; 0.1% methanol in ethanol may be readily detected, corresponding to about 0.01 grm. of methanol in the amount taken for the test.

Another one of the official methods of the A. O. A. C. is that of Trillat (*Analyst*, 1899, **24,** 13). This method requires preliminary fractionation with Glinsky bulb tubes and the oxidation of the first fraction with potassium dichromate and sulfuric acid and the subsequent treatment with dimethylanilin and oxidizing agents to produce a colored derivative. This test is even more difficult of application than the Riche and Bardy method and does not give as satisfactory results nor is it quite as delicate.

In the United States Pharmacopeia, 8th revision, the Mulliken-Scudder test (*Am. Chem. J.*, 1899, **21,** 266) was made official. In this method the sample was oxidized by repeated immersions of a copper wire spiral which was heated to redness in a non-luminous flame. The resulting mixture of aldehydes, in a sample containing both ethanol and methanol, was heated slightly to drive off the major portion of the more volatile acetaldehyde and the identification of formaldehyde by the test with resorcinol and sulfuric acid. This method was not very delicate, a proportion of less than 2% usually escaping detection. Among the unimportant modifications of this test which are no longer used are those of Prescott (*Pharm. Archives*, 1901, **4,** 86) and Haigh (*Pharm. Rev.* 1903, **21,** 404).

From the time of the appearance of the Mulliken-Scudder test to the present, most of the efforts of investigators along this line seem to have been confined to the development of the same fundamental idea, *i. e.*, converting the alcohol into an aldehyde and the recognition of the methyl derivative, formaldehyde, by one of its characteristic tests. In 1903 Thorpe and Holmes (*J. Chem. Soc.*, **83,** 314) proposed an oxidation method with potassium dichromate and estimation of the resulting carbon dioxid in the case of methanol, ethanol yielding no carbon dioxid under the conditions of the test.

A few years after the copper spiral method of oxidation had been proposed, the Sanglé-Ferrière-Cuniasse test (*Ann. Chim. Anal.*, 1903, [1], **8,** 82) was published, in which the oxidizing agent suggested was potassium permanganate and the phloroglucinol test was used for the detection of the formaldehyde.

Shortly after this method appeared, Scudder and Biggs (*J. Amer. Chem.*

Soc., 1905, **27,** 892 and 1906, **28,** 1202) made several important contributions to the literature of the subject, reviewing all of the principal tests thoroughly and pointing out their respective advantages and defects. It was stated by these authors that in the presence of powerful oxidizing agents even ethyl alcohol would give rise to small amounts of formaldehyde and that it was necessary to carry out all tests under carefully controlled conditions as regards amount of oxidizing agent, time of oxidation and temperature at which the oxidation was allowed to take place. This is especially true of the Voisenet method (*Bull. Soc. Chim.* 1906, [3], **35,** 748), in which a chromic acid mixture is proposed as the oxidizing agent and which has been shown to yield traces of formaldehyde from pure ethanol even in the cold.

It is probably ignorance of this fundamental fact that has been responsible for the reporting by some investigators of the presence of methyl alcohol in liquors where none is likely to be found, *e. g.*, beers.

Another oxidizing agent was proposed by Hinkel (*Analyst*, 1908, **33,** 417) who employed ammonium persulfate and relied for the subsequent detection of the formaldehyde upon the well known morphin-sulfuric acid reaction.

During the fifteen years that have elapsed since Hinkel's paper was published, most of the methods proposed have been modifications or variants of one or the other of the oxidation methods just reviewed. Among the important references in the literature during this period may be mentioned the following:

Vorisek (*J. Soc. Chem. Ind.* 1909, **28,** 827); Deniges (*Compt. Rend.* 1910, **150,** 832); Wirthle (*Chem. Zeit.* 1912, **36,** 700); Koenig (*Chem. Zeit.* 1912, **36,** 1025); Bono (*Chem. Zeit.* 1912, **36,** 1171); Raikow (*8th Int. Cong. Appl. Chem.* 1912, **25,** 417); Schmiedel (*Pharm. Zeit.* 1913, **54,** 709); Manzhoff (*Zeit. Unt. Nahr. Gen.* 1914, **27,** 469); Rinck (*Zeit. Unt. Nahr. Gen.* 1914, **28,** 98); Blanksma (*Chem. Weekblad* 1914, **11,** 26); Wilks (*Wellcome Trop. Res. Lab. Bul. Chem. Sec.* 1914, **1,** 5); Pazienti (*Ann. Chim. Applic.* 1915, **3,** 279); Reif (*Abs. Kais. Ges.* 1915, **50,** 56); Fendler (*Zeit. Unt. Nahr. Gen.* 1916, **30,** 228); Salkowski (*Zeit. Unt. Nahr. Gen.* 1916, **36,** 262); Schryver and Wood (*Analyst* 1920, **45,** 164); Hoton (*Ann. Fals.* 1920, **13,** 490) and Chapin (*J. Ind. Eng. Chem.* 1921, **13,** 543); Lyons (*J. Amer. Pharm. Assoc.* **12,** 1923, 323); Kraemer (*J. Amer. Pharm. Assoc.* 1923, **12,** 506); Meurice (*Ann. Chim. Anal.* 1923, [2], **5,** 204).

In the Schmiedel and Manzhoff methods it is proposed to convert the

radical into nitromethanilin; in the Meurice method, which I have found very unsatisfactory, a physical separation is proposed by means of a strong solution of ammonium sulfate. All of the other methods are based upon oxidation of the alcohol by one of the oxidizing agents previously mentioned, and usually consist in varying the method of testing for the resulting formaldehyde.

Excellent reviews and criticisms of various methods have been published by G. Cecil Jones (*Analyst* 1915, **38,** 218); A. O. Gettle (*J. Biol. Chem.* 1920, **43,** 211) and Jos. W. E. Harrisson (*Proc. Penna. Pharm. Assoc.* 1920, **9,** 204).

In the 9th revision of the United States Pharmacopeia, which was issued in 1916, there was adopted a modification of the Deniges test (based on the Sanglé-Ferrière-Cuniasse method), which was given as follows:

"Dilute the alcohol with distilled water until it contains 10 per cent. by volume of ethyl alcohol. Transfer 5 mils of the dilute alcohol to a test tube, add to it 2 mils of a potassium permanganate solution (made by dissolving 3 grm. of potassium permanganate in 100 mils of distilled water), and 0.3 mil of sulfuric acid, and allow the mixture to stand for five minutes. Now dissolve the precipitate of manganese dioxid by the addition of sulfurous acid, drop by drop with agitation, then add 1 mil of sulfuric acid and 5 mils of fuchsin-sulfurous acid T. S. and mix them. After standing ten minutes, a colorless liquid results (*methyl alcohol*)."

The foregoing test is intended to be applied to ethyl alcohol of 95% strength. It may, however, be adapted to all kinds of distillates suspected of containing methyl alcohol if the concentration is brought to the proper degree by fractionation or dilution. In the presence of methyl alcohol a violet coloration appears in the final liquid, which color is proportionate in its intensity to the amount of methyl alcohol present.

This test was severely criticized after it had appeared as an official test, many observers stating that all kinds of interfering colorations were given by pure ethyl alcohol. In 1919, Ehman (*Amer. J. Pharm.*, 1919, **91,** 594) reviewed the criticisms, studied the test and found that the difficulty occurred on account of directing the addition of the fuchsin-sulfurous acid immediately after the strong sulfuric acid was added, without control of the rise of temperature, or directions to cool before adding the final reagent. Ehman suggested that the temperature of the liquid should be brought down to 25° C. before adding the fuchsin-sulfurous acid T. S., and those who constantly employed the test found his observations and recommendations to be correct.

In consequence of the criticism of Ehman, the test as revised and proposed for adoption in the 10th revision of the United States Pharmacopeia reads as follows:

"Dilute the alcohol with water to contain about 5% by volume of ethyl alcohol. To 5 c.c. of this diluted alcohol contained in a test tube add 0.5 c.c. of phosphoric acid and 2 c.c. of a 3% aqueous solution of potassium permanganate and allow the mixture to stand for ten minutes. Add 1 c.c. of an aqueous 10% solution of oxalic acid and let stand until the solution is clear brown. Now add 1 c.c. of sulfuric acid, cool to about 25° C., add 5 c.c. of fuchsin-sulfurous acid T. S., mix well and allow to stand for ten minutes. At the end of this time the solution, when observed against a white background, may have a reddish or pale green color, but not a distinct blue or violet (*methyl alcohol*)."

This test, like the one quoted from the previous edition of the United States Pharmacopeia, is intended to be applied to official ethyl alcohol of about 95% strength. It may be adapted as a routine laboratory test if the proper concentration is obtained before proceeding with the test.

In both the original and the revised United States Pharmacopeia tests the alcohol is highly diluted (to 10% of alcoholic content in the original and 5% in the revised test). The oxidation is produced by the addition of a carefully regulated volume of a 3% aqueous solution of potassium permanganate. In the original test the oxidation was allowed to proceed for five minutes; in the revised test the time is lengthened to ten minutes. The oxidation takes place in a slightly acidulated liquid (in the former case sulfuric acid is used and in the latter phosphoric acid is directed). The clearing away of the colored compounds of manganese is accomplished in the original case by the cautious addition of sulfurous acid solution, which entirely discharges the color, and in the revised test by the addition of 1 c.c. of a 10% aqueous solution of oxalic acid which changes the liquid to a clear brown, the color being finally discharged by the acidulation with 1 c.c. of sulfuric acid, which is directed in both tests, in the latter case the warning being given to reduce the temperature to 25° C. before proceeding further.

If methyl alcohol is present there will be found in the solution at this stage of the procedure, formaldehyde as well as acetaldehyde, which is present as the corresponding oxidation product of ethyl alcohol. In the official application of the test from this point use is made of a test which is not specific for

formaldehyde but is a test for aldehydes in general. The inhibition of the reaction for acetaldehyde is accomplished by the acidulation with sulfuric acid to a proper degree to accomplish this result.

A careful study has been made of the several factors of importance in the application of the test and to determine the approximate degree of sensitiveness, if possible. Comparisons were made throughout the work, of both the old and the new U. S. P. tests. After working with both for a few days the advantage of the latter over the former was recognized, and a way was found to still further obviate the possibility of overheating the mixture when the sulfuric acid is added just before the final stage of the test and to avoid the necessity of cooling as directed. This was accomplished by the simple and satisfactory expedient of employing a sulfuric acid solution which had already been diluted to a point at which it no longer heated when diluted still further.

The matter of the dilution of the alcohol was first studied to determine whether the 10% or the 5% dilution is preferable. There seems to be little or no choice in this matter, except that in the 10% dilution the color comes up a little more intensely. In the matter of sensitiveness none of the variations was capable of detecting with any degree of certainty a smaller proportion of methanol than one part in five hundred parts of ethanol. This corresponds to about 0.001 grm. of methanol in the amount taken for the test by the U. S. P., IX, and about half this amount by the proposed revised test.

The sensitiveness of the fuchsin-sulfurous acid T. S. was then tested out upon solutions of formaldehyde of known strength and was found to respond to 1 in 10,000 within five minutes and to 1 in 100,000 within twenty minutes.

The failure to detect smaller quantities of methanol than was experienced in practically carrying out the test is undoubtedly due to a failure to oxidize the methyl alcohol in the presence of a great excess of ethyl alcohol or the possibility that it may be completely oxidized to formic acid and thus destroyed for the practical purposes of the test.

Some experiments were then conducted to ascertain the effect of larger proportions of the oxidizing agent and the effect of longer periods of oxidation as well as of increased temperature during oxidation. These experiments showed that increasing the time of oxidation up to as much as one hour had no effect upon the final result except to increase the sensitiveness of the test; that increasing the proportion of oxidizing agent to more than double the

amount directed (5 c.c. instead of 2 c.c. of the 3% aqueous solution of potassium permanganate) caused pure ethyl alcohol to react positively toward the test, and that heating with the permanganate solution invariably caused the production of formaldehyde from pure ethyl alcohol.

Instead of using the fuchsin-sulfurous acid reagent in the final determination a number of the other tests for formaldehyde were employed. The resorcinol test and the Leach test were found satisfactory within certain limits but less sensitive than the official test. The modifications of the phenylhydrazin test were found to be unsatisfactory, either on account of the interfering substances resulting from the preliminary treatment or because they were so delicate as to give positive reactions even with pure ethanol.

The conclusions drawn after conducting many hundreds of experiments over a period of several months, during which abundant opportunity occurred to try out various proposed methods with unknown samples of commercial origin, are that the test as proposed in the U. S. P., IX, is very satisfactory when the precaution is observed of reducing the temperature of the liquid prior to adding the fuchsin-sulfurous T. S., but that the modifications as proposed for the U. S. P., X, especially in the use of oxalic acid instead of sulfurous acid for dissolving the precipitated manganese oxid, are improvements over the original test.

A still further improvement has been made in which the necessity of reducing the temperature of the liquid during the test is obviated. The proposed test is carried out as follows:

Dilute the alcohol with water to contain about 5% by volume of ethyl alcohol. To 5 c.c. of this diluted alcohol, contained in a graduated test tube of 20 c.c. capacity, add 5 or 6 drops of phosphoric acid and 2 c.c. of a 3% aqueous solution of potassium permanganate and allow the mixture to stand for ten minutes. Add 1 c.c. of an aqueous 10% solution of oxalic acid and allow it to stand until the liquid is a transparent brown. Now add 5 c.c. of a previously diluted and cooled sulfuric acid (which has been diluted in the proportion of 3 volumes of water to 1 volume of acid), add 5 c.c. of fuchsin-sulfurous acid T. S., mix well and allow to stand for ten minutes.

At the end of this time the solution when observed against a white background should not show a distinct blue or violet tint (*methyl alcohol*).

The use of graduated test tubes greatly simplifies the operation as a routine test and the employment of the previously diluted and cooled sulfuric

acid enables the operation to be carried out with greater certainty and expedition.

A method of making the record of the test permanent which has also been found to be of advantage is to dye the color on strips of white wool, following the customary procedure as in the dyeing test for coal tar colors.

The test is sensitive to 1 part of methanol in 500 parts of ethanol. If smaller proportions of methanol are present or suspected, a preliminary fractionation may be carried out and the test applied to the 1 c.c. fraction coming over first from a 10 c.c. sample. In this manner positive reactions were obtained with as small amounts as 1 part of methanol to 10,000 parts of ethanol. Smaller proportions than this would be without practical significance.

CHEMICAL ATTRACTION

DAVID WILBUR HORN

MANY solid compounds decompose yielding gaseous decomposition products, provided the temperature of the compounds is elevated sufficiently. Deville (1857) designated this process *dissociation*. A compound need not be a solid in order to exhibit such dissociation, and it has been suggested that a substance possibly need not be a compound in order to dissociate. Lockyer regarded it probable that the elements not recognizable in the sun's spectrum have been "*dissociated* by intense heat." It is stated that the hydrogen of the chromosphere is "too hot to burn," the temperature of the solar surface being above that of dissociation, *i.e.*, so high that all compounds of hydrogen would there be decomposed.

Confining attention to isolated exothermic compounds, it is a reasonably fair generalization that temperature and stability bear an inverse relation to each other. If it be postulated that stability is a measure of the chemical attraction by which the constituents of a compound are held together to form the compound, then (other things being equal) it is a reasonably fair generalization that relative temperatures of dissociation of comparable compounds bear a direct relation to the relative strengths of the chemical attraction, or, of the resultant of all the attractive and repulsive forces at work, within each of the compounds considered.

The problem suggested in this generalization is complicated, and it may be that our knowledge of chemical attraction and of heat is too meager to permit of its solution. In the matter of chemical attraction our knowledge has been advanced far enough along certain lines to permit of some ordinal arrangements of the acids. In the matter of heat, changes in amount and temperature are capable of exact quantitative statement.

To the general problem no novelty can attach. The works of Thomsen (1853 forward) and Berthelot (1865 forward) are generally familiar. In this connection, however, it is the writer's thought that in certain cases of dissociation there is a field for study of great interest and promise. What is offered in this paper has to do mainly with a method of attack. The experiments

described are all more or less preliminary and illustrative, rather than final, in character.

The equilibria that establish themselves when any solid compound undergoes thermal dissociation into another solid and a gas, may be represented diagrammatically by a curve similar in shape to the curve shown in Figure 1. For each temperature a certain and single-valued pressure is sooner or later established simultaneously with equilibrium. Similarly, the equilibria that establish themselves between a liquid and its vapor are equally well represented diagrammatically by the same curve.

The distinction between the phenomena within the system represented by the equation $CaCO_3 \rightleftarrows CaO + CO_2$, for example, and the phenomena within the system $\underset{\text{Liquid}}{H_2O} \rightleftarrows \underset{\text{Vapor}}{H_2O}$ must be regarded as conventional. It has been convenient to refer to the former as a *chemical change* and the latter as a *physical change*. For the present purposes this distinction is unnecessary and misleading. The facts as set forth by the common curve suggest a close analogy between these two kinds of processes, which is not contemplated when they are distinguished as *chemical* and *physical* respectively. This suggested analogy was much strengthened when Horstmann (1869) showed that dissociation may be dealt with (mathematically) in the same terms as vaporization. Not long afterward Willard Gibbs (1875–1878) demonstrated that this analogy must be regarded only as a special case under a perfectly general mathematical mode of treatment, rigidly applicable to all equilibria in heterogeneous systems. The potential usefulness to chemists of the point of view developed by Gibbs was pointed out by Roozeboom (1887) in a paper in which he arranged the dissociation-equilibria then known according to the number of components in each system and the number and character of the phases involved. The Phase Rule is a simple and useful expression of Gibb's great generalization.

Under the Phase Rule, which is ordinarily written $F = C + 2 - P$, both the systems exemplified by dissociating calcium carbonate and by evaporating water, respectively, are *monovariant*. In the systems like calcium carbonate, there are 2 components (here CaO and CO_2) and 3 phases (here solid $CaCO_3$, solid CaO and vapor CO_2); if these numbers are substituted for C and P respectively in the Phase Rule, the result is $F = 2 + 2 - 3 = 1$. In the systems like water, there is 1 component (here water) and 2 phases (here liquid water, and vapor water); if these numbers are substituted for C and P respectively in the Phase Rule, the result is $F = 1 + 2 - 2 = 1$. When the

degrees of freedom, F, in any system equals 1, it can exhibit only monovariance; for each temperature at equilibrium there will be one and only one pressure, and vice versa, no matter how complex the system may seem to be.

With the analogy between the two orders of monovariant systems in mind, there is justification for comparing the corresponding curves throughout their lengths. When any set of curves representing comparable dissociating systems are compared along a line of constant temperature, the systems arranged in order of the magnitude of the pressures within each at this common temperature will be thereby arranged in the order of their relative stabilities. The least stable system will exhibit the highest pressure, and vice versa. Also when any set of curves representing comparable dissociating system are compared along a line of constant pressure, then the systems arranged in order of the temperatures within each at this common pressure will be thereby arranged in the order of their relative stabilities. The least stable system will exhibit the lowest temperature, and vice versa. This idea the writer has presented previously (*Amer. Chem. Jour.*, 1908, **39,** 222), and has shown that by such comparisons among cuprammonium compounds he was led to an arrangement of the acids in an order that is substantially the same order as that previously arrived at (by Thomsen) through thermo-chemical considerations, and (by Ostwald) through considerations of volume.

With the analogy between the two orders of monovariant systems in mind, there is also justification for comparing or seeking to compare the phenomena represented at the termination of the curves. For water and for all other liquids that withstand decomposition and that have been studied, the graph for this monovariant system of the first order (that is, one-component system) reaches an end in the region of *critical phenomena*. A temperature, called the critical temperature, is ultimately reached above which it is impossible to obtain or to retain the liquid (Andrews, 1869). It is fair to say that at the critical temperature the resultant of all the forces of attraction and repulsion at work within the liquid reaches a value that if exceeded the exhibition of properties as a liquid is impossible no matter how great the concentration thereafter realized within the system. By analogy, the interesting suggestion arises that at the upper end of the dissociation curve a temperature may be reached above which it will be impossible to obtain or to retain the compound that has been dissociating. This temperature would be the one at which the resultant of all of the forces of attraction and repulsion at work within the

compound (by virtue of which it becomes and remains a compound) reaches a value which if exceeded becomes such that the properties of the compound can not be exhibited no matter how great the pressure (that is, concentration in the vapor phase) thereafter realized within the system. In so far as this analogy has value, it offers a mode of attack upon "chemical affinity" by which a numerical (quantitative) statement may be found within reach.

As already stated, what is offered in this paper is preliminary and illustrative, rather than final, in character, and it has to do mainly with the experimental method of attack. In the simplest case the apparatus need consist only of two concentric test-tubes; within the inner tube the dissociating solid is placed with a thermometer mounted with its bulb in this solid. The outer tube is used to furnish an insulating air-jacket. The inner tube connects with the space into which the vapor from the dissociation is to escape through a delivery tube or other outlet so arranged as to permit and to compel the escape of the gas at constant pressure. Upon immersing the apparatus in a thermostat at a temperature such as to produce dissociation, it will be noticed that the thermometer in the solid will register at first a rapid rise, then a gradual halt, and then a slight drop to a temperature that will be found to remain constant for an hour or more although it may be several degrees lower than the temperature of the thermostat. The readings on the thermometer are made at regular intervals of time (every half-minute or every minute) and the readings may be plotted as ordinates against the time intervals as abscissas. The appearance of such graphs was pointed out by the writer some time ago (Amer. Chem. Jour., 1907, **37,** 619), and may be seen in Figure 2 in this paper.

Each of the constant temperatures realized in a properly conducted experiment may be taken as the abscissa of the point on the dissociation curve (such as that shown in Figure 1) of which the pressure fixed in the experiment is the ordinate. By fixing the pressure (ordinates) at different values in a series of experiments, upon the same dissociating substance, the corresponding temperatures (abscissas) may be learned; and the data for plotting the dissociation curve thus become available. Such a dissociation curve is shown in Figure 1, plotted upon the data set forth in Table 1. In obtaining these data I was assisted by Miss Ida V. McWilliams.

The plan of fixing the pressure and then learning by experiment the corresponding temperature of equilibrium is in flat contrast with the conventional procedure. Conventionally, the temperature is fixed and the pressure

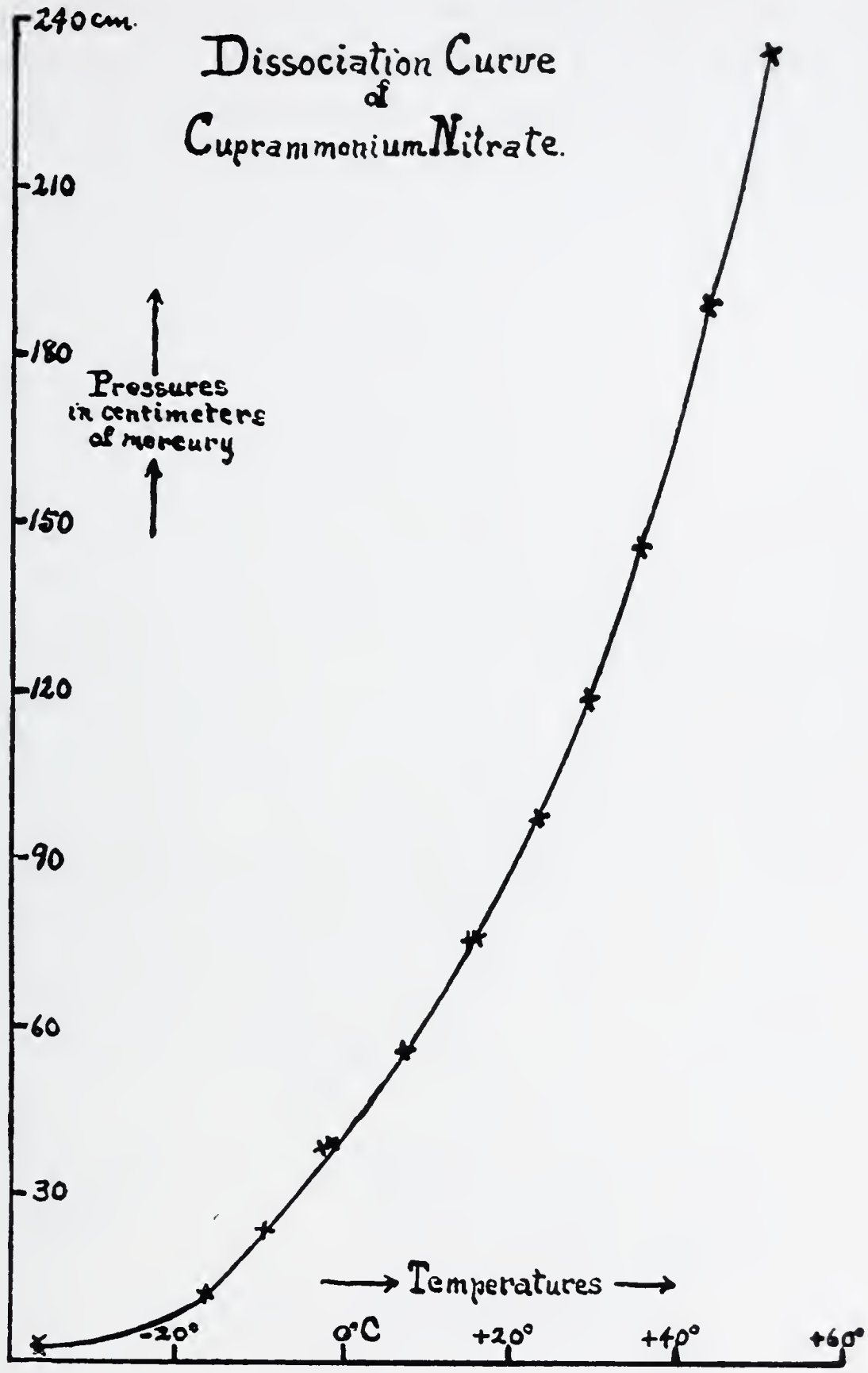


FIG. 1

TABLE I.—DISSOCIATION OF CUPRAMMONIUM NITRATE

Pressure in cm. of mercury	*Temperature in degrees centigrade*	*Pressure in cm. of mercury*	*Temperature in degrees centigrade*
2.2	−36.5	75.7	+15.4
3.5	−36.0	75.8	+15.8
12.0	−16.3	97.4	+23.4
24.0	− 9.4	119.5	+29.3
38.7	− 2.5	146.0	+35.4
39.1	− 1.5	190.0	+43.4
54.9	+ 7.9	234.7	+50.5

learned experimentally. The method discussed has such advantages as economy of time, much greater flexibility, and more convincing results. It may be called a *dynamic* method in contrast with the conventional *static* method, because the equilibrium temperature is learned while the compound is actually continuously dissociating; in the static method, the equilibrium pressure is attained and measured only after dissociation has reached equilibrium.

That the dynamic method will yield the same result as the static appears from experiment upon ammonia-silver chlorid. Isambert's determinations by the static method of the dissociation pressures of ammonia-silver chlorid at various temperatures show a value of 19.9° C. at 1 atmosphere; Horstmann's independent determinations by the static method show 19.05°. The dynamic method in my hands showed at 1 atmosphere pressure that a temperature of 19.4° was attained and maintained constant by the system to within 0.2° for upward of one hour. The period of constant temperature may be shortened or lengthened at will, at any pressure, by decreasing or increasing the mass of dissociating solid experimented upon.

No one familiar with the Phase Rule would venture to oppose this proposed method by urging that it is applicable only to the few ammonia-metal compounds studied. The following systems have been studied and found to exhibit the characteristic behavior to be expected under the Phase Rule of all monovariant systems consisting only of vapor and solid phases:

$$2\{AgCl \cdot 3NH_3\} \rightleftarrows \{2AgCl \cdot 3NH_3\} + 3NH_3$$
$$\{Na_2B_4O_7 \cdot 10H_2O\} \rightleftarrows \{Na_2B_4O_7 \cdot 5H_2O\} + 5H_2O$$
$$\{CuCl_2 \cdot 2H_2O\} \rightleftarrows \{CuCl_2\} + 2H_2O$$
$$2NaHCO_3 \rightleftarrows Na_2CO_3 + H_2O + CO_2$$
$$\{Cu(NO_3)_2 \cdot 6NH_3\} \rightleftarrows \{Cu(NO_3)_2 \cdot 4NH_3\} + 2NH_3$$

The data for the cuprammonium nitrate system are given in Table 1 and Figure 1. The sodium hydrogen carbonate system has been studied differently with results set forth in Figure 2.

The general course of a time-temperature curve in a successful experiment is shown in Figure 2 by the graph marked 80°. The phenomena to be observed in a dissociating system whose equilibrium is disturbed by some external influence, such as heat at a higher temperature in my experiments, can in general be predicted by what is commonly called the Theorem of Le Chatelier, which may be stated (Ostwald) as follows: "If a system in equilib-

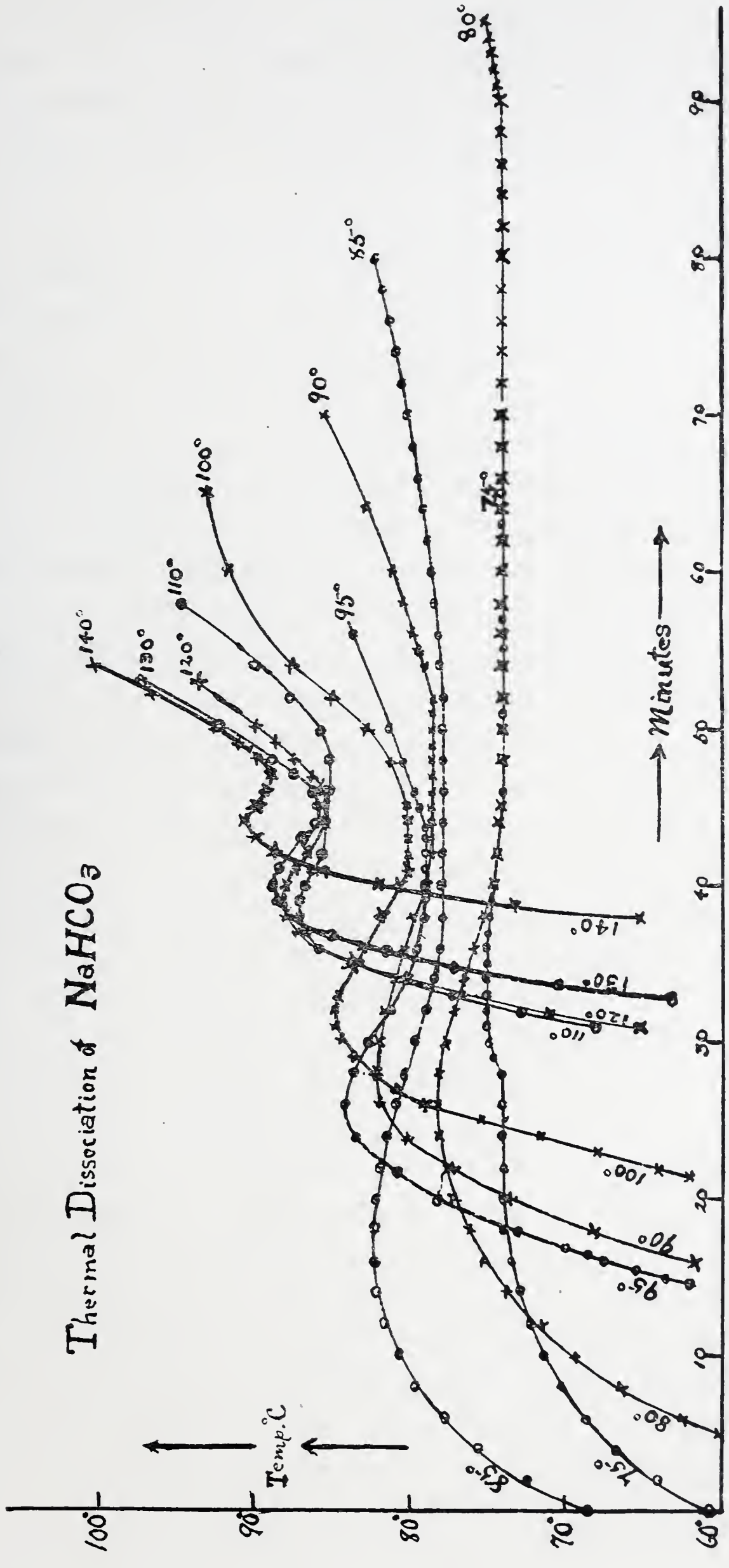

Thermal Dissociation of $NaHCO_3$
Temp. °C
Minutes
100°
90°
80°
70°
60°
10
20
30
40
50
60
70
80
90
140°
130°
120°
110°
100°
95°
90°
85°
80°
75°
65°

FIG. 2

rium is subjected to a constraint by which the equilibrium is shifted, a reaction takes place which opposes the constraint. . . ." The humps to be observed in the graphs in Figure 2 which give to each an S-shaped appearance, I have thus far assumed to represent a phenomenon of *continuity* (James Thomson, 1871).

These dissociating systems seem to differ from such systems as those composed of melting solids or boiling liquids in that the velocity at which the change can occur is less. One cannot overheat a melting solid, perhaps, or a boiling liquid (unless the other phase be absent), but one can overheat a dissociating solid. A moment's consideration of the well-known case of α-sulphur and β-sulphur will serve to show that such slowness is to be expected in change in solids. If α-sulphur is heated slowly enough, it will pass at 96° to β-sulphur, and then only will its temperature rise higher than this transition point. But if it is heated rapidly, its temperature will rise to 115° at which the α-sulphur will melt. Failure to recognize the existence of a finite rate of transition may lead to overheating and to misinterpretation of the minimum temperature maintained within the system.

The experiments made upon sodium hydrogen carbonate illustrate this point. In obtaining these data I was assisted by Mr. Charles E. Gulezian. The temperatures written at the ends of each curve are the temperatures of the thermostat during each experiment.

The transition temperature of the system $2NaHCO_3 \rightleftarrows Na_2CO_3 + H_2O + CO_2$ at 2.5 cm. pressure is approximately 74° C. Even when the system is overheated excessively, as when the thermostat temperature was set at 140°, the system still shows the behavior predictable by the Theorem of Le Chatelier, although the transition-velocity is too low to permit of the realization of the true transition temperature for the fixed pressure. As the degree of overheating is reduced by lowering the temperature of the thermostat, the behavior of the system approaches more and more closely to normal. If the reader will study these curves, he will see that the minimum temperature and the maximum duration of constancy are approached as the thermostat-temperature comes nearer to the transition temperature. The general rule for procedure would seem to follow, namely, set the thermostat temperature at a few degrees in excess of the supposed transition temperature; the difference between the two should be such that the system exhibits the phenomena of *continuity* to an extent greater than could be accounted for by the experimental error.

If one transition-point at a given pressure is established, the general procedure thereafter is less time-consuming. And when a few points on the dissociation curve are known, its slope may be estimated closely enough to guide the further experiments.

The phenomena that I am dealing with are undoubtedly phenomena confined to the surface of the solid and regions contiguous thereto. Only at the surface of the solid where contacts with the vapor phase (and the other solid phase) occur can an equilibrium set up. The end of the period of constant temperature on a time-temperature curve such as the curve for $NaHCO_3$ marked 80° does not therefore correspond to complete decomposition of the $NaHCO_3$ present. It corresponds to a condition in which so much of the $NaHCO_3$ on the surfaces of the solid particles has been decomposed into Na_2CO_3 that the change can no longer continue at a sufficient velocity successfully to "oppose the constraint." The change in weight of any of the dissociating systems, or the analysis of the residue, or the microscopic examination of the solid particles in the residue will confirm this statement. If one selects colored crystals, for example $CuCl_2.2H_2O$ (green-blue), which on dissociation yield a solid of another color, for example $CuCl_2$ (brown), then a direct microscopic examination of the residue will show at a glance what has actually occurred in one of these experiments. The brown powder stands out in strong contrast to the underlying green-blue crystals.

The extension of the dynamic method throughout wider ranges of a dissociation-curve is limited only by the experimenter's ingenuity and resources. The experiments described for the first time in this paper along with those referred to in previous papers form a reasonably complete experimental survey of the dynamic method.

TRANSACTIONS OF THE WAGNER FREE INSTITUTE OF SCIENCE

OF

PHILADELPHIA

VOL. XI

1927

WAGNER FREE INSTITUTE OF SCIENCE
MONTGOMERY AVE. AND SEVENTEENTH ST.
PHILADELPHIA

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BIOCHEMICAL STUDIES OF THE NORTH AMERICAN SARRACENIACEAE

By

JOSEPH SAMUEL HEPBURN, A.M., B.S. IN CHEM., M.S., PH.D.

FRANK MORTON JONES, F.E.S.

and

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CONTRIBUTIONS FROM THE CONSTANTINE HERING RESEARCH LABORATORY, HAHNEMANN MEDICAL COLLEGE OF PHILADELPHIA

INTRODUCTION

THE observations and experiments forming the basis of the following papers on the North American *Sarraceniaceæ* had their beginning in 1892 when *Sarracenia flava*, *S. rubra*, and *S. purpurea* came under observation in Richmond County, North Carolina. In succeeding years all the species have been under frequent observation in their native habitats, and have been made the subject of several papers on insect-plant relations by one of us (Jones).

Field and laboratory experiments on the biochemistry of the *Sarraceniaceæ* were commenced in 1917, and have been continued until the present time. These researches form part of a general study of insectivorous plants, work on *Nepenthes* having been begun by Hepburn and St. John in 1914. *Dionaea* has been made the subject of a recent paper.

Observations and field experiments have been made or material for laboratory examination collected chiefly near the following localities for each species:

Darlingtonia californica.—Keddie, Plumas Co., and Mt. Eddy (the type locality), Siskiyou Co., Cal.

Sarracenia minor.—Summerville, Berkeley Co., S. C.; Jacksonville, Duval Co., Fla.

Sarracenia Sledgei.—Mobile, Theodore, and Bayou La Batre, Mobile Co., Ala.; Biloxi, and Wiggins, Harrison Co., Miss.

Sarracenia flava.—Southern Pines, Moore Co., Hamlet, Richmond Co., and Wilmington, New Hanover Co., N. C.; Summerville, Berkeley Co., S. C.; De Funiak Springs, Walton Co., Fla.; Bay Minette, Baldwin Co., Ala.

Sarracenia Drummondii.—De Funiak Springs, and Freeport, Walton Co., Fla.; Bayou La Batre, and Theodore, Mobile Co., and Bay Minette, Baldwin Co., Ala.

Sarracenia rubra.—Southern Pines, Moore Co., and Hamlet, Richmond Co., N. C.; De Funiak Springs, Walton Co., Fla.

Sarracenia purpurea.—Tolland, Tolland Co., Conn.; Whitings, and Toms River, Ocean Co., N. J.; Pocono Pines, Monroe Co., Pa.; and many other localities from Maine to Mississippi.

Sarracenia psittacina.—De Funiak Springs, Walton Co., Florida; Bayou La Batre, Mobile Co., and Bay Minette, Baldwin Co., Ala.; and Ocean Springs, Jackson Co., Biloxi, and Wiggins, Harrison Co., Miss.

Samples of pitcher liquor and aqueous solutions of the nectar, which were collected in the field for laboratory examination at Philadelphia, were preserved by addition of 0.2 percent of trikresol. The collection of material for bacteriological study and for chemical examination of the plant tissues is described in detail in the papers on these respective subjects.

In this series of papers the authors have followed Macfarlane[95] in the nomenclature of the *Sarraceniaceæ*. They are indebted to Dr. Macfarlane for many helpful suggestions in the course of these researches.

The authors record their indebtedness to the Franklin Institute of the State of Pennsylvania for permission to use portions of a paper on absorption of nutrients and allied phenomena in the pitchers of the *Sarraceniaceæ*, originally published in the *Journal of the Franklin Institute*, February, 1920. The Institute has also permitted the reprinting of several illustrations from its *Journal*.

The American Museum of Natural History has granted permission to reprint three illustrations from its journal, *Natural History*.

WORK OF PREVIOUS INVESTIGATORS ON THE BIOCHEMISTRY OF THE SARRACENIACEÆ

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D.

DIGESTION AND ABSORPTION

MACBRIDE[1] studied *Sarracenia adunca* (*S. minor, S. variolaris*) and *S. flava* in their native habitat. His observations were made chiefly during 1810 and 1811 and were communicated to the Linnean Society of London in 1815. He noted the masses of captured insects within the pitchers and wrote: "What purposes beneficial to the growth of these plants may be effected by the putrid masses of insects, I have never ascertained."

Hooker[2,3] found "a slight acid secretion" in young pitchers of *Darlingtonia californica*. He states that the pitchers of both *Sarracenia variolaris* (*S. minor*) and *S. flava* secrete a fluid. However, the pitchers of *S. variolaris* examined by him contained no secretion. He also examined both half-grown and full-grown pitchers from cultivated plants of *S. flava;* they contained no fluid except what may have been accidentally introduced. According to Hooker, secretion of "water" by the pitchers of *S. purpurea* had never been observed, and he suggested "the possibility of this plant either having no proper secretion of its own, or only giving it out after the pitcher has been filled with rain water." He considered it quite likely that the "digestive functions" in *Sarracenia* pitchers may be of short duration. Digestion experiments were not made with the *Sarraceniaceæ*.

Mellichamp[4] made observations on *Sarracenia variolaris* (*S. minor*) growing in its native habitat in the vicinity of Bluffton, South Carolina. Unopened pitchers usually contained from 3 to 5 drops of liquor, occasionally as many as 10 drops, rarely 15 drops. The liquor was bland and somewhat mucilaginous to the taste; it left an astringent taste in the mouth. Perfect, open pitchers usually contained from 10 to 15 drops of liquor, very rarely a half-drachm. Mellichamp adds: "*I have, however, since found the fluid much increased in quantity*, very frequently a drachm, and *sometimes as much as two*

drachms." He states that the secretion of pitcher liquor "appears to continue during the whole period of the entrapment, which I suppose does not last over two or three weeks at most."

"During this active period the 'water' is attracted to the top of the mass and permeates and percolates through every portion of it so that eventually all the soft parts of the insects are thoroughly dissolved. After these animal juices have been partially or entirely absorbed by the plant, or by the larva of the Sarcophaga fly which continues to grow and fatten upon this rich diet; the remaining portion, commences gradually to dry, until only the backs and legs and shells of the various insects remain."

The digestive action of the pitcher liquor was tested on venison, apparently *in vitro*, without a bactericide. Bits of fresh venison were immersed in the pitcher liquor and in a corresponding amount of pure water. After the lapse of 15 hours, the venison in the pitcher liquor was more changed, softened, and broken up, and far more offensive to the nostrils (being offensive to a disgusting degree) than was the venison in the water. Mellichamp concluded that this experiment perhaps showed that the liquor hastened the decomposition of the insects and their conversion into "liquid manure."

Edwards[5] considered that the pitcher liquor of *Darlingtonia californica* did not possess true digestive power, but was able to "cause decomposition" of the prey. He stated: "I do not attempt to speak authoritatively upon the subject, but I am inclined to think that no process similar to digestion goes on within the plant, but that the fluid mass derived from the decay of the imprisoned insects descends through the tube into the earth, and is taken up by absorption, through the roots, thus acting as a kind of liquid manure. It is true that in the dead leaves the hard integuments of insects, such as the elytra of beetles, and the bodies of wasps and hornets are to be found undecayed, but this may be because the liquid secreted by the plant is not powerful enough to cause decomposition of these parts before the plant itself decays." He commented on the "most disgusting" smell of the pitcher contents, and recorded that "after handling a number of specimens of the tubes, it is necessary to use some disinfectant like ammonia or chloride to remove the disagreeable odor."

The statements of Canby[6] concerning *Darlingtonia californica* were based in part on the observations of his correspondent, Lemmon. Canby mentions the secretion of a pitcher liquor by *Darlingtonia*. "Several inches

of the bottom of the tube are filled with a clear fluid (secreted by the leaves it must be). . . . Mr. Lemmon has kindly sent me an ounce phial completely filled with the fluid 'from two petioles' . . . It is scarcely necessary to say, that as it is certain no water can get into the tube by any ordinary means, and as the fluid is always present in healthy leaves, it must be secreted by the plant as Mr. Lemmon says."

Canby also mentions the offensive odor of a patch of *Darlingtonia* plants whose pitchers contained prey. "Mr. Lemmon further says 'I came upon a patch once in September and smelled it from afar so offensive was it. A portion of the leaves filled with insects to the depth of four to six inches, had fallen down apparently from the weight of the fluid and insects.'"

The response of *Darlingtonia californica* to food stimulation was discovered by Mrs. R. M. L. Austin who studied these plants in their native habitat, Butterfly Valley, Plumas County, California. Mary E. Pulsifer Ames[7] mentions Mrs. Austin's researches, and publishes the following extract from a letter received by herself from Mrs. Austin. "In July, 1875, I fed a great many of the leaves, some with fresh raw mutton and others with that which was boiled. The liquid, in the course of a week, would fill the tubes and flow out of the orifice." Asa Gray[22] states that Mrs. Austin, studying *Darlingtonia californica*, found that "the watery liquid in the pitcher, which must be wholly a secretion, is much increased in quantity after the capture of insects."

Batalin[8] described certain changes in the cell-wall of the pitcher-lining of *Darlingtonia californica* and of several species of *Sarracenia* after prey had been captured. He concluded that these changes facilitated the absorption, by both genera, of the products derived from the captured insects.

Pitchers of green-house plants of *Sarracenia flava* never contained any liquor, although their inner surface was frequently moist. The outer surface of the pitcher always was green. The inner surface of the lower part of the pitcher was green when insects had not yet been captured, and became a brownish yellow at those places where captured insects adhered to the wall. This change affected only the interior surface of the pitcher, and occurred only in its lower or detentive zone. Sections of the inner surface of this zone were cut. When the inner surface was green and free from adhering insects, the cavity surface of the epidermal cells was quite smooth, homogeneous, and free from markings of any kind. When insects were adhering to the epidermal cells, the cavity surface of each cell contained one or two paler areas surrounded

by a more or less broad, intense yellowish-brown margin which lay at a higher level. The epidermal cells are provided with a cuticula which forms the outermost layer of the cell membrane. In cells, to which insects had adhered, the cuticula had become detached from the paler colored areas; the margin was the remaining portion of the cuticula. Cells, which adjoined the altered cells but were free from insects, had not lost any of their cuticula and were free from paler areas.

Some sections revealed the manner in which the cuticula was detached. Bubbles appeared, as if some substance were secreted, between the cellulose membrane and the cuticula. The bubbles were of a brighter yellow than the cuticula. The secreted substance, the nature of which is unknown, gradually collected in greater amount, produced a further separation of the cellulose membrane from the cuticula, rendered the latter yellow and viscous or gelatinous, and finally caused detaching of the cuticula. At times not only the cuticula, but possibly the entire cuticular layer was cast off from the paler areas. The secreted substance owed its origin to a stimulus exerted on the epidermal cell by the adhering insect. Occasionally the cuticula was detached from as many as ten areas on a single cell; these areas were then surrounded by a yellow network of the remaining cuticula. The detentive hairs spring from cells which are smaller than those participating in these changes.

In *Sarracenia purpurea*, the change in the cuticula was almost the same as in *S. flava*. In the lower portion of the wide-open pitcher, each of the altered cells lost the cuticula from an oval area or from two areas. In the narrower portion of the pitcher, near its bottom, the cuticula might be cast off from as many as ten or twelve areas on a single cell and, at times, might become detached from a number of adjacent cells as a thin unbroken membrane which showed distinctly the contour of the individual cells.

In old pitchers of *Sarracenia variolaris* (*S. minor*) which had not captured insects, the cuticula was not homogenous in the lower or detentive zone, but was extremely thin or entirely lacking on certain regions, especially on the clefts which occurred at the outer edge of the cuticula where it met the inner border of the projection of the side wall of the cell. These clefts consisted of two or three rows of extremely small points or irregular, four-cornered, rounded areas, or of a single row of larger areas; occasionally these larger areas united into two or three still larger areas, between which were smaller areas or points. Other points and areas of this type occurred on the surface of the cuticula.

When the cuticula was cast off, detachment usually took place along these clefts so that only narrow strips of cuticula remained as scollops. At times, a cell would lose the cuticula from two or three isolated areas instead of a single large area. The remnants of the cuticula were usually yellow, and apparently gelatinized; the cuticula-free surface of the cell and its side walls were almost colorless.

Only old pitchers of *Darlingtonia californica* were available for study. In the detentive zone, the epidermis resembled that of *Sarracenia variolaris*. The areas, on which the cuticula was extremely thin or entirely lacking, were similar to those of *S. variolaris* with respect to both shape and distribution; occasionally they were so strewn over the entire surface of the cell that it resembled a sieve-plate. The cuticula was thus modified only in those parts of the pitcher cavity which contain long, stiff hairs. These modified areas were almost entirely lacking in the upper region with its short thin hairs. Clefts of large surface area predominated in the middle portion of the pitcher. In the lower, narrow portion, the modified areas were points, scattered over the cuticula in such large numbers that its surface was granular. Detachment of the cuticula could occur in all parts of the cavity of the pitcher; it was cast off from the entire surface of the cell along the line of the modified areas; only a remnant of cuticula was left between adjoining cells.

Zinc chloride plus iodine was of service as a stain in study of the sections.

Batalin drew the following conclusions from his research: "The physiological significance of the phenomena here described is quite clear. They can be considered as processes which facilitate for the plant the absorption of dissolved substances from without. It has been known for a long time that the cuticula is that part of the epidermis membrane which offers the greatest resistance to the penetration of substances into the cell. Therefore one can look upon its detachment as an adaptation for facilitating this entrance of substances into the plant. Accordingly in these two genera we have an interesting example of adaptation of the plants for particular purposes. . . . In *Sarracenia* and *Darlingtonia*, in consequence of the almost complete lack of glands in the pitchers, their function is here taken over by the entire inner surface; the epidermis is changed for this purpose in such a way that the absorption of solutions is rendered possible. From the described process, the manner and way in which the cuticula is cast off, it is seen that a substance is secreted between it and the cellulose membrane. How Nature has provided

this secretion is unknown to me; it is not improbable that it contains the solvent (Lösungsmittel) for the digestion of the proteins."

"These pitcher plants must be grouped with those which satisfy their nitrogen requirement through the captured insects. . . . They take up the nitrogen which they require directly from the insects . . . and not indirectly by manuring the soil on the surface of which the dead leaves plus the captured insects decay."

Schimper[9] studied *Sarracenia purpurea* growing wild on the Massachusetts coast. When the plants grew in the open, the pitchers usually contained water, i. e., liquor. Observations made on plants under cultivation led to the conclusion that a very small portion of this liquor is secreted by the pitcher itself; the major portion owes its origin to the rain. Secretion of liquor occurs in young pitchers, indeed long before opening. Acid-reacting droplets occur on the lower hairy region as well as on the middle region of smooth epidermis, and collect at the bottom of the pitcher.

Digestion experiments were made; small pieces of meat were introduced into several pitchers; other pitchers were permitted to catch insects. A control experiment was made at the same time by introducing small pieces of meat into water contained in a glass vessel. The meat dissolved very slowly in the liquor within the pitchers; in fact, the rate of solution did not exceed that in the control experiment; hence it was concluded that the pitcher liquor did not contain pepsin. Moreover, the presence of bacteria was definitely shown. Introduction of nitrogenous compounds did not increase the acidity of the pitcher liquor.

Schimper stated: "Innumerable worms were present in all the leaves studied, these possibly participate in the transformation of the animal bodies into soluble compounds." This may be construed to mean that the larvæ of the insect associates possibly play a part in the digestion of the prey.

The decomposition products of the prey were absorbed. This was definitely shown by certain changes which occurred in the epidermis cells in the bottom portion of the pitcher, and, to a lesser degree, in the cells of the adjacent subepidermal layer. As already stated, some pitchers were nourished by captured insects or by introduced meat. Other pitchers were, in a sense, starved by plugging the mouth with tissue paper as soon as the lid had opened. A striking difference was observed between the nourished and the starved pitchers. The cell sap of the cells in question is rich in tannin; it formed one,

two, or more very strongly refractive, glittering drops in the nourished pitchers; in starved pitchers it formed, at the most, a single drop which possessed these properties to a very slight degree and occupied a much larger space. The drops were not suspended in the cell sap, but represented the entire cell sap. Food caused a marked removal of the chlorophyll granules from the cell wall and their occurrence about the vacuoles or drops. The substances of animal origin in solution in the pitcher liquor acted as a stimulus; the protoplasm acquired greater power of imbibition, and withdrew water from the cell sap. The colloidal tannin was unable to pass through the protoplasm by osmosis and remained in the cell sap, which thus became more concentrated and acquired a higher refractive power, while the protoplasm became less refractive. These phenomena were not produced exclusively by nitrogenous compounds, but were obtained when either a dilute solution of sodium chloride, or sea water, or borax was used as a stimulus. Pure water was without stimulating action.

The "nourished" appearance was true of the cells at the base of the hairs even before the pitcher lid had opened. It was frequently noted at the border of a section in cells which had either been cut or rested directly on cut cells, and was then due to the disorganization of the cell contents. Therefore only somewhat thick sections were of value.

Schimper concluded that absorption certainly occurs through the entire interior surface of the hairy bottom portion of the pitcher. The thick-walled hairs have no function other than preventing the escape of the prey. If the pitchers contain very little liquor and many insects, the surface tissues become brown and decomposed. Pitchers of this type, which are extremely rare in plants growing in the open, apparently served Batalin in his studies. The property of the protoplasm to attain a greater degree of capacity for imbibition under the stimulus of certain substances appeared to be, in all likelihood, of direct significance for the nutrition of the plant. The very marked swelling would produce a widening of the micellar pores and a marked increase in the diosmotic properties of the protoplasm; as a result, the entrance into the tissues of the substances contained in the pitcher liquor would at least be facilitated, possibly be first made possible. Therefore the insect captures, in all probability, are actually of value to the plant.

Higley[10] made an extensive series of analyses of the pitcher liquor of *Sarracenia purpurea*, growing in the open, probably in Wisconsin.

His findings would indicate that the water content of the pitcher liquor is collected rain. "As a result of the examination of over 800 leaves I find that none contained any fluid before they had opened. . . . After opening there is no fluid till after the first rain except in a few cases when there has been a heavy dew."

One hundred analyses were made in order to ascertain the composition of the pitcher liquor before it contained any insects. Twenty-five of these analyses are published and may be summarized. The samples of pitcher liquor were collected from 2 to 20 days after the pitcher had opened to such an extent that rain could easily enter it, and from 1 to 18 days after rain had fallen. As to color, 13 samples were clear, 1 slightly yellow, 6 yellow, 3 tinged, and 2 dirty. The amount of solids and acids and bases, which were present, are reported in the table. The organic solids "consisted, to a great extent, of pollen, various other vegetable structures, Infusoria, algæ and the like." The reaction was "nearly neutral" in 4 samples, "slightly acid" in 11 samples, and "acid" in 10 samples.

CHEMICAL CONSTITUENTS OF THE PITCHER LIQUOR OF *SARRACENIA PURPUREA*, ACCORDING TO HIGLEY.

			Liquor prior to capture of insects.	*May samples.*	*June samples.*	*July samples.*	*August samples.*
Number of analyses published			25	10	10	10	10
Solids, parts per 1000	Organic..	Maximum	20	130	173	207	260
		Minimum	2	60	120	137	164
	Inorganic	Maximum	5	8	13	12	18
		Minimum	1	2	5	6	8
Number of samples containing	Ammonium*		25	10	10	10	10
	Sodium		12	9	10	10	10
	Potassium		24	10	10	10	10
	Magnesium		2	5	10	10	
	Calcium		19	8	10	10	10
	Aluminium		6	3	10	5	8
	Iron		4	3	7	7	10
	Silica		1		3	7	4
	Carbonates		25	10	10	10	10
	Chlorides		18	10	10	10	10
	Sulphates		9	3	10	5	8

On the last day of each month—May, June, July, and August—100 samples of pitcher liquor were collected, and analyzed; for each month, 10 typical analyses, including the extremes, were published. The data concerning solids and occurrence of acids and bases are summarized for each month in the table.

* A trace of nitric acid was probably present as ammonium nitrate in these samples.

The May samples were gathered from 1 to 23 days after rain had fallen. The reaction was acid in all 10 of the published analyses; the color of the liquor was "yellow" in 2, "dark" in 2, "dirty" in 3, and "wine" in 3 samples.

The June samples were collected from 1 to 7 days after rain had fallen; the reaction was acid in the entire 10 published analyses; 1 sample was "wine" color and 9 samples "dirty."

The July samples were obtained from 3 to 15 days after rain had fallen; the entire 10 published analyses were characterized by an acid reaction and a "dirty" color.

The August samples were collected from 5 to 20 days after rain had fallen; all 10 published analyses showed an acid reaction and a "dirty" color.

The pitchers, from which the liquor was procured for analysis, represented the growth of the season up to the date of collection. Each pitcher was cut open, and the contents were used only when they were of such consistency that they flowed easily.

The acidity of the pitcher liquor became greater each month, and was especially marked during July and August. "To just what acid, if any particular one, the reaction was due in the liquid of the earlier pitchers is not certain, but in the last two months both malic and citric acids appeared, the former in greater abundance."

The source of the malic acid is indicated: "It seems highly probable that the first lot of insects merely decay after maceration in the water first collected in the pitchers and that this mass acts not only as a stimulant to further decay but also to render the liquid more capable of absorbing certain organic principles from the leaf, such as Malic acid, which aid in the preparation of the abundant supply of food for absorption by the leaf. Thus the first mass might be called a digestive excitant."

Absorption occurred more rapidly during the last days of June and the months of July and August than earlier in the season; the cells then became filled with absorbed matter.

Ammonia was quite readily absorbed by the pitcher from the liquor. Several sets of experiments were made. In each set use was made of two pitchers of the same age and size, growing on the same plant, containing the same volume of liquor and approximately the same amount of insect remains; the pitchers chosen contained but a few insect remains. A quantitative determination of the "organic ammonia" was immediately made upon the fluid from

one of the pitchers. The other pitcher was so turned and propped that rain could not enter, and was left thus for a week; the "organic ammonia" content of its pitcher liquor was then determined, and compared with the amount found in the first pitcher. "The fluid showed a decided decrease in each case, from the amount found in the one used in comparison. Though these analyses were perhaps quite far from sure in every detail, yet the average difference on comparison, viz.: sixty parts in one hundred would indicate quite rapid absorption, for such an amount could not possibly be removed in any other way." Therefore the pitchers absorbed nitrogenous compounds derived from "the decomposing remains of the insects."

Chemical examinations were made of the soils upon which the plants were growing. Not all the inorganic salts which were found in the pitcher liquor could be accounted for as from the soil.

Zipperer[11] found that the pitcher liquor of *Sarracenia purpurea* corroded starch grains after several days' action, and dissolved coagulated egg white in a short time. These results indicated that both a diastase and a peptonizing enzyme are secreted into the pitcher. Zipperer also made experiments with isopods of the genus *Oniscus*, either *Oniscus scaber* or *O. murarius*. The pitcher liquor first exerted a narcotic action on the prey; then death occurred as a result of suffocation; and finally, of the entire body, only a residue of chitin armor remained. He concluded that *Sarracenia purpurea* is a "finely developed insect trap, in the interior of which the insect is killed by a secretion, and assimilated by ferments."

Goebel[12] demonstrated that water and various solutions, introduced into *Sarracenia* pitchers, decreased markedly in volume in a few days. The water or solution was introduced into a pitcher of *Sarracenia illustrata* (a hybrid between *S. flava* and *S. purpurea*) until the level of the pitcher contents was 10 centimeters below the orifice; the latter was closed with a cork; and paraffin of low melting point was poured over the cork to prevent evaporation of the water. The level was marked by a strip of paper pasted on the exterior surface of the pitcher. The pitchers were examined 48 hours later with the following results:

One pitcher had received 20 cc. of 1 percent solution of formic acid and some swollen fibrin; the decrease in volume, due to absorption, was 6.8 cc.; the remaining solution still had an acid reaction; the fibrin appeared entirely unattacked.

Another pitcher had received 10 cc. of water, of which 2 cc. were absorbed.

A third pitcher had received 10 cc. of very dilute meat infusion exactly neutralized with sodium carbonate; the decrease in volume was 2.5 cc.; the meat infusion became alkaline in reaction and cloudy, and was full of bacteria.

A similar experiment was made with a pitcher of *Sarracenia Drummondii*. A 5 percent peptone solution, which had been introduced into the pitcher, underwent a decrease of somewhat more than 7 cc. in volume; the remaining solution occupied a volume greater than 2 cc., was markedly cloudy, and possessed a faint odor of putrefaction.

A young, green pitcher of *Sarracenia purpurea* was used in another experiment. A piece of meat the size of a barley grain and 10 cc. of water were introduced into the pitcher; and the orifice was closed airtight. Two days later 2.8 cc. of the water had been absorbed; the piece of meat was scarcely attacked, it was not foul, though thickly covered with bacteria.

Pitchers, each of which had received 5 cc. of meat juice and a small fragment of meat, were characterized 3 days later by a foul odor and the presence of ammonia. Therefore, whenever the pitchers contain a sufficient quantity of substances which are capable of putrefaction, that phenomenon makes its appearance.

The plants used in these experiments had previously been kept rather moist, in a cool north room. Under other conditions, the absorption would have been greater.

These conclusions are drawn. *Sarracenia* pitchers secrete neither a proteolytic enzyme nor a substance which prevents putrefaction. Digestive glands are not present. The inner surface of the pitcher, especially its lower portion, is able to absorb water and substances dissolved in the latter. Absorption occurs through the inner wall of the pitcher. The plant, as a rule, is but little sensitive to putrefaction products, provided they do not form in the pitchers in too great a quantity.

Goebel states that secretion of a pitcher liquor has never been observed in cultivated specimens of *Darlingtonia*. He suggests that the liquor of pitchers of plants growing in their native habitat may possibly exert an antiputrefactive action, provided too many insects are not captured. This genus was included in the absorption tests. A fragment of meat the size of a barley grain and 5 cc. of distilled water were introduced into each of three young pitchers of *Darlingtonia*. The orifice of each pitcher was then closed as tightly

as possible with cotton. Two days later the greater portion of the fluid had been absorbed; a portion had evaporated; the remaining fluid contained bacteria and moulds, but did not have a putrid odor. However, an odor of putrefaction was distinctly noticeable in the dead prey of other pitchers.

The presence of larvæ amid the prey in pitchers of *Sarracenia* and *Darlingtonia* was considered by Goebel as evidence that digestion of the prey does not occur in these two genera as rapidly as in *Nepenthes*. The identity of the compound, produced by digestion of the prey and absorbed by the pitcher wall, is not known. From experiments on *Nepenthes*, it is very probable that ammonia is absorbed. The specific cause of the digestion, whether autolysis of the prey or the activity of micro-organisms, is not stated.

Goebel concluded that *Darlingtonia* like *Sarracenia*, does not secrete a proteolytic enzyme in its pitchers. Secretion of a substance, which prevents putrefaction, does not occur in either genus, except, possibly, to a slight extent.

Lambert[13] made experiments on the secretion of pitcher liquor by *Sarracenia purpurea*, and on absorption from the pitchers of that species. His work apparently was done on the isle of Saint-Pierre and on the coast of Newfoundland.

The plants used in the secretion tests were kept under normal conditions. The liquor was withdrawn from the pitchers by means of a fine pipette; and every trace of moisture was removed with bibulous paper. The plant was placed beneath a shelter to exclude rain water, and the soil was watered amply. The interior of the pitchers remained perfectly dry.

Neither the introduction of several drops of ether nor the movements of a living insect produced any secretion of liquor by the pitchers. It had been hoped that these stimuli would cause secretion of fluid by the "stomach zone" into the dry pitcher cavity.

These results showed that the pitcher by itself does not secrete any fluid.

In the absorption tests, several drops of a solution of a crystalloid stain, such as methylene-blue of fuchsin, were added to the pitcher contents. Approximately 2 hours later the pitcher was cut open. In the pitcher wall of the bottom region, absorptive zone, or "stomach," the interior epidermis and one or two layers of subepidermal "digestive cells," which are characteristic of this zone, were stained blue or red according to the dye used. Staining of the pitcher wall occurred only in this zone. Colored colloids, *e. g.*, tincture of cochineal, were not absorbed and did not stain the tissues.

A highly concentrated solution of methylene-blue was left in living pitchers for an entire week. Only the epidermis and digestive cells were stained. The result was the same at the end of several hours as at the end of the week. After absorption, the stain was localized; and a limit was thereby placed on the total amount of absorption.

Lambert evidently considered that enzymic digestion of the prey, and absorption of the products occurred in the pitchers. "The albuminoid mass formed in the pitcher by the cadavers of the drowned insects will be attacked little by little by the liquid contained in the wall of the stomach, a liquid which passes through the wall as through a dialyzer. Under the action of this pepsin-like liquid, the albuminoids will be transformed into peptones and rendered assimilable, *i. e.*, will then be able to pass through the thin membrane of the epidermal cells in the stomach region and of the digestive cells which localize them."

The liquor is constantly agitated by living worms or annelids (larvæ?) in pitchers containing prey. It is suggested that this agitation takes the place of peristalsis in promoting digestion and absorption of the prey.

In the researches of Fenner[14] on insectivorous plants, *Sarracenia flava* was the only species of the *Sarraceniaceæ* studied. In their normal condition, the pitchers contained no liquor. Secretion of a digestive fluid occurred after insects were present in the pitcher. Flies were introduced into a young pitcher in sufficient number to fill the lowermost part of the pitcher, and were pressed together so that the mass was in intimate contact with the wall of the pitcher. In the course of 2 or 3 hours, a slight amount of mucilaginous secretion was poured out from the pitcher lining, and digested the insect bodies which were in immediate contact with the lining. The products of digestion were absorbed. Each newly captured insect shoves together the cadavers already present, and again brings them into direct contact with the pitcher lining and the secretion. The mass of insect remains therefore is well moistened and thoroughly saturated with the secretion. When the insect remains were present only in the absorptive zone, putrefaction was entirely absent. When the amount of prey was so great that the absorptive zone was filled, and a large number of insects were present in the detentive zone, then a very distinct odor of decay emanated from the latter zone; this phenomenon occurred chiefly in older pitchers.

Certain changes in the cell-contents were attributed to absorption of the

products of digestion. When cadavers of freshly captured insects were present in a pitcher, the cells in the bottommost or absorptive zone were characterized by a typical aggregation and turbidity of their contents, due to absorption of organic substances. These changes were most marked in the innermost layer of the pitcher wall. Another indication of absorption was the frequent occurrence of a large number of dark masses in the second layer of cells of the pitcher lining in the absorptive zone.

Several tests were made of the absorptive power of the pitcher. A small volume of water was introduced into a pitcher so that its level did not extend above the absorptive zone; the water was absorbed in 2 or 3 days.

When a larger volume of water was used, absorption produced a slight sinking of its level, but soon ceased. Introduction of insects into such a pitcher was followed by development of a putrefactive odor within 4 to 6 days. The dilution of the secretion with much water had unfavorably influenced the digestive function of the absorptive zone.

Several drops of meat juice were introduced into a very young pitcher; they disappeared after a time, even though the orifice of the pitcher had been closed with a cotton plug to prevent evaporation. Absorption therefore took place. However, if the pitcher were half-filled with meat juice, and the level of the latter extended far above the absorptive zone, an odor of putrefaction appeared at the end of approximately 10 days. These experiments showed that the absorptive power is limited.

Fenner concluded that *Sarracenia flava* is an insectivorous plant, provided with a digestive enzyme, requiring and utilizing its prey for its nutrition, and able to absorb animal substances through a definite, though small, region of the pitcher.

Robinson[15] conducted greenhouse experiments on plants of *Sarracenia purpurea* which had been gathered recently at Poughkeepsie, New York, and near Lakewood, New Jersey. Various substances were introduced into the pitchers, usually as aqueous solutions; and the changes in both the pitchers and the introduced substances were noted.

"Before a solution was placed in a pitcher the contents were withdrawn by means of a pipette, the pitcher was thoroughly, though gently rinsed with tap-water and distilled water, and swabbed with absorbent cotton. After the solution had been placed in the pitcher, it was covered with lace net." Insects were thereby kept out of the pitchers; an additional precaution to

exclude ants from the pitchers was to place the crocks, containing the plants, in water.

Solutions, introduced into the pitchers, produced the following results:

A 0.5 percent solution of acetic acid caused pitchers to wither above the level of the liquid within a few hours; the pitchers were dead at the end of 6 days.

A $\frac{1}{1024}$ molar solution of potassium nitrate did not injure the pitchers in which it was kept, with frequent renewals, for 6 weeks. A 0.5 percent solution of this salt was not injurious; in one experiment, perceptible growth occurred in its presence. With a 1 percent solution, the pitchers withered in 6 days; with a 2 percent solution, they became dry and brown in 3 days. Both young and mature pitchers behaved in the same manner.

Sach's nutrient solution, each liter of which contained 1.0 gram calcium nitrate, 0.25 gram potassium nitrate, 0.25 gram dipotassium phosphate, 0.25 gram magnesium sulphate, and a trace of ferrous sulphate, caused pitchers "to begin to decay within a few days, the tissues being entirely dead in from two to three weeks."

A dilute solution of Liebig's meat extract produced partial withering of the pitcher in less than a week; and complete decay occurred in approximately 2 weeks.

Milk was diluted, 1 drop in 10 cc. of distilled water; the resulting solution was neutral to litmus; it remained odorless and neutral in reaction after 6 days in the pitcher. When the concentration of the milk was doubled, acidity developed, and the pitcher decayed almost completely in 2 weeks. With a still higher concentration of milk (20 percent by volume), the pitcher contents coagulated and acquired an unpleasant odor within 2 days. "It was inferred that the pitcher gave out an alkaline substance which reacted with the acid produced in the very dilute solution of milk but was not sufficient to neutralize the solutions of greater strength. There was nothing to indicate that the milk fat or protein was digested."

When a 10 percent solution of glucose was kept in the pitchers for periods varying from 4 days to 3 weeks, it retained the power to reduce Fehling solution on heating, and the presence of much carbohydrate was shown by the α-naphthol test. Some of the glucose underwent ordinary fermentation, but the products were without apparent detrimental influence on the plant.

Sucrose was used in concentrations between less than 1 percent and 33⅓

percent; no bad effect was noted. The 33⅓ percent solution did not injure the pitcher during a period of 2 months; young pitchers containing this solution grew at the same rate as those containing distilled water. After sucrose solutions of various concentrations had been in the pitchers from 3 to 7 days, they gave a reddish precipitate of cuprous oxide with hot Fehling solution, and also spontaneously produced a heavy reduction of that reagent without heating. Blank, or control, experiments on the reagents and on water, which had been kept in the pitchers, gave no reduction. Therefore the sucrose had been cleaved into invert sugar.

Starch paste was kept in the pitchers for from 3 to 13 days: it then reduced Fehling solution, but only on boiling: and it still gave a blue color with iodine. In some experiments toluene was introduced into the pitchers with starch paste; sufficient toluene was used to form a layer above the surface of the starch paste; the contents of these pitchers also acquired the power to reduce Fehling solution. Reduction of Fehling solution was never obtained in control experiments made on the reagents, and on tap-water plus toluene which had been permitted to stand in the pitchers.

Neutral olive oil was mixed intimately with either distilled or tap water, in the ratio of 0.4 cc. of oil and 9.6 cc. of water. The mixture was introduced into pitchers; in some experiments no bactericide was used, in others toluene was added as a bactericide; the results were the same in the two series of experiments. From 4 to 7 days later, the contents of the pitchers were removed and titrated with 0.01 molar potassium hydroxide solution, using phenolphthalein as an indicator. Control experiments were made on portions of the mixture which were not introduced into pitchers. The titrations showed that the oil had not been hydrolyzed by the pitchers.

Ethyl butyrate was also used as a reagent for a fat-splitting enzyme. Tap water, or 0.01 molar potassium hydroxide solution, or 0.01 molar acetic acid solution was kept in the pitchers for 1 day. The liquid was then removed and mixed with ethyl butyrate, 4 drops of the ester to 2 cc. of the liquid. After incubation at room temperature for 24 hours, the liberated butyric acid was titrated with 0.01 molar sodium hydroxide solution, using phenolphthalein as an indicator. Control experiments were made *in vitro* with the ester and portions of the standard solutions. The results showed the absence of enzymic cleavage of the ester.

Distilled water produced no change in the external appearance of pitchers in which it was kept, with frequent renewals for approximately 5 weeks.

In another series of experiments water was kept in the pitchers for 6 days, then removed, and tested for the presence of a protease. The water from the pitchers was divided into several portions, to each of which a granule of fibrin was added. The test was carried out in neutral, in slightly acid, and in slightly alkaline solution; each of these three sets of tests was made both in the presence and in the absence of toluene. "The result was quite uniform, for the fibrin granule remained apparently unchanged in each liquid."

The general conclusions reached by Robinson were:

"1. The pitchers of *Sarracenia purpurea* can adapt themselves to solutions of very different osmotic strengths.

"2. They give out an enzyme which hydrates sucrose and starch to reducing materials, presumably simple sugars.

"3. They have no fat-digesting power.

"4. They do not secrete a protein-dissolving enzyme."

Hepburn, St. John, and Jones[18] made a preliminary report on the presence of a protease in the liquor of both unopened pitchers and open pitchers of *Sarracenia flava*, on the bacterial sterility of the contents of unopened pitchers of *S. flava* and *S. minor*, and on the types of bacteria present in the contents of open pitchers of these two species.

In another paper, Hepburn, St. John, and Jones[19] made a preliminary report on the biochemistry of the pitcher liquor of the North American *Sarraceniaceæ*, and on the response by the pitchers to introduced substances. They also gave a detailed account of the absorption of various nutrient solutions which were introduced into the pitchers.

Certain flies of the genus *Sarcophaga* pass their entire larval stage within the *Sarracenia* pitchers, living on the cadavers of the captured insects. Hepburn and Jones[20] demonstrated the presence of antiproteases in these larvæ; this indicates that the proteases, which occur in the pitcher liquor, exert a digestive action on proteins present within the pitcher cavity.

Burnett[21] wrote of the pitchers of *Sarracenia:* "The water in these receptacles, impregnated by the half-decomposing animal matter, doubtless affords a highly nutritive and invigorating diet to the plant," and "the Sarraceniæ, if kept from the access of flies, are said to be less flourishing in their growth, than when each pouch is truly a sarcophagus." He mentioned digestion of the prey, and spoke of the pitchers as special organs "for the especial purpose of retaining food, and absorbing thence its nutritious particles." He

considered the pitchers the nearest approach in the vegetable kingdom to the stomach of animals. Burnett apparently believed that the prey underwent digestion within the pitchers, and that the products of digestion were then absorbed and used for the nutrition of the plant.

Wherry[94] has commented on the reaction (hydrogen-ion concentration) of the pitcher liquor of *Sarracenia purpurea*.

Values obtained by Wherry on the pitcher liquor of this and other species of *Sarracenia* have been placed at the disposal of the authors, and are given on page 69.

PHYSIOLOGICAL ACTION OF THE PITCHER LIQUOR AND OF THE NECTAR

The Pitcher Liquor

The physiological action of the pitcher liquor on insects was studied by Mellichamp.[4] Liquor was collected from pitchers of *Sarracenia variolaris* (*S. minor*). Insects were placed in a layer of the liquor which was not deep enough to immerse them completely. House-flies were used in approximately 20 experiments; they became anesthetized or intoxicated in from 0.5 to 10 minutes. When removed from the liquor, they recovered; the time required for recovery varied from 0.5 hour to one hour or longer. The liquor also exerted this action on a cockroach, a moth, and a common house spider. "Without doubt, therefore, the secretion found in the tubes of *Sarracenia variolaris* is intoxicating, or narcotic, or anesthetic, or by whatever word we may prefer to indicate that condition to which these small insects succumb." The liquor quickly saturated, clung to, and clogged the wings of house-flies, rendering flight impossible. Pure water does not exert this wetting action, but "runs" from the wings.

The following experiment showed that the pitcher liquor does not evolve a poisonous exhalation for overcoming the prey. Two house-flies were suspended in a cage of thin gauze within a large wide-mouth phial, which contained approximately one-half ounce of pitcher liquor; about 8 hours later, the flies were still struggling frantically to escape.

In a second communication on the stupefying action of the pitcher liquor of *Sarracenia variolaris* (*S. minor*), Mellichamp[16] wrote: "Pour out a teaspoonful or two of the fluid in an ounce measure or a small wine-glass.

Throw in a fly so that his wings will be wet or slimed. He will in a few minutes cease to struggle and will appear as if dead. Take him out after a while and let him dry, and in about half an hour he will revive."

Pitcher liquor was collected at a time when no rain had fallen for nearly two weeks, and, in part, from unopened pitchers; it was turbid, slightly acid in reaction, had very little, if any, taste, and was very active with respect to stupefying power.

A sample of pitcher liquor, which had been kept for 3 years, was clear, neutral in reaction, and without much sediment; it was nearly or quite inert with respect to the stupefying power.

Watson[17] repeated the experiments of Mellichamp, and confirmed the latter's results on the stupefying power of the pitcher liquor of *Sarracenia variolaris* (*S. minor*).

Zipperer[11] conducted experiments on the action of the pitcher liquor of *Sarracenia purpurea* on isopods of the genus *Oniscus*, and concluded that the liquor first exerted a narcotic action on the isopods, and that their death was due to suffocation.

From experiments made by Lambert[18] on millipeds, it appeared that the pitcher liquor of *Sarracenia purpurea* had no action on *living* animal organisms.

The Nectar

Mellichamp[4] permitted flies, large red ants, and smaller ants—both black and red—to feed on the nectar of *Sarracenia variolaris* (*S. minor*). The nectar was innocuous, and entirely without narcotic, stupefying, or intoxicating action on these insects.

This conclusion was confirmed by later experiments of Mellichamp,[16] made on pitchers (leaves) of this species. "While still fresh, the upper portions of these leaves were cut off and slit open, thereby exposing the honeyed secretion on the internal surface, which was very abundant and glistening, sweet to the taste and viscid to the touch. These were then flattened out on a large newspaper, the whole surface of which was covered with them. Many house flies were soon attracted and commenced to feed, and I carefully watched their motions without any interruption for the space of one hour. The result was precisely as previously stated. In no instance did I discover the slightest unsteadiness or tottering in any of the flies, although I watched some of them

feeding at one spot for at least ten minutes, at the expiration of which time they flew off apparently unhurt. They continued feeding and flying off from the leaves during the hour I watched them, and certainly not one fell, nor was there any indication at any time of either stupor or intoxication." The pitchers, which were used, were secreting nectar freely. The experiment was repeated twice, and the same result was always obtained. The nectar functioned simply as a lure.

CHEMICAL COMPOSITION OF THE TISSUES

Porcher[23] made various qualitative tests upon the cold infusion, the decoction, and the alcoholic extract of the rhizomes of *Sarracenia flava* and *S. variolaris* (*S. minor*). Meconic acid, morphine, narcotine, and quinine were not found in the rhizomes. Volatile, bitter and astringent principles are mentioned: "The bitter and astringent principles are volatile, for upon boiling a portion of the powdered root, the liquid was almost tasteless."

Porcher included in his paper the results obtained by Shepard in a preliminary examination of the dried rhizomes of the species mentioned. Sections of the rhizomes imparted an acid reaction (red color) to moistened blue litmus paper. Upon incineration, a white ash was obtained containing silica, calcium carbonate, and potassium carbonate. From qualitative tests, made chiefly on the extract in 80 percent alcohol and the extract in water acidulated with sulphuric acid, Shepard concluded that the rhizomes contained liguin, coloring matter, traces of a resin, an acid salt of calcium "(the acid being neither the tannic nor the gallic, but possibly one altogether new), and a salt of some alkaloid, related perhaps to cinchonia, which, should it prove new, may be called Sarracenia."

Both Porcher and Shepard noted the pigment. Shepard obtained a rich red precipitate (color of port wine) on the addition of potassium carbonate to the solution obtained by extraction of the rhizomes with water acidulated with sulphuric acid. The color was attributed to the pigment of the rhizomes. Porcher described the extraction of pigment from the rhizomes by spirits of ammonia with the production of a solution of blood red hue.

Björklund and Dragendorff[24, 25] made an exhaustive study of the chemical composition of the dried tissues of *Sarracenia purpurea*. The rhizomes and the pitchered leaves were studied separately. The various chemical compounds were separated from each other and then identified by appropriate

tests; and quantitative determinations were made of the amount of each compound present. The specimens examined apparently had been collected in the spring of the year.

The percentage composition of the tissues, as reported by these investigators, is given in the table. In addition to the compounds there enumerated, they state that the following substances were present in quantities "not determinable":

PERCENTAGE COMPOSITION OF THE DRIED TISSUES OF *SARRACENIA PURPUREA* ACCORDING TO BJÖRKLUND AND DRAGENDORFF

Constituents	*Rhizomes*	*Pitchers (Leaves)*
Hygroscopic moisture	12.08	8.60
Cellulose	19.82	14.55
Starch	25.55	..
Lignin, cuticular substance, and insoluble plant mucilage	3.17	19.90
Soluble plant mucilage	0.89	..
Sugar	9.56	3.95
Soluble plant albumin	5.70	1.02
Insoluble plant casein	7.10	1.40
Volatile amide	0.18	0.77
Volatile acid (acrylic acid)	1.49	0.12
Indifferent white resin	8.81	5.47
Wax	0.10	0.53
Ash	2.25	2.14
Silica	0.21	0.31
Ferric and phosphoric oxides	0.91	0.50
Calcium carbonate	0.09	..
Potassium sulphate	0.25	0.64
Sodium chloride	0.45	0.03
Magnesium carbonate	0.03	..
Sodium sulphate	0.15	..
Calcium sulphate	...	0.71

In the *rhizomes:* (1) an unknown substance which, on boiling in aqueous solution, furnishes a substance similar to cinchona red; (2) a non-volatile acid; (3) a tannic acid similar to caffetannic acid; (4) amorphous extractives; (5) a chromogen which yields a red pigment on treatment with hydrochloric acid, and (6) a volatile aromatic substance with an odor similar to that of "Rad. Carlinæ."

In the *pitchers (leaves):* (1) a non-volatile acid; (2) tannic acid; (3) plant mucilage soluble in boiling water; (4) non-crystalline extractives; (5) a red

pigment soluble in dilute hydrochloric acid, and (6) traces of carbonates and magnesium.

The volatile amide, which occurred in both the rhizomes and the pitchers, is also termed an alkaloid, but lacked the toxic properties of a true alkaloid as was shown by feeding it on bread to a mouse. It was a liquid at ordinary temperatures; its molecular weight was approximately 35; its odor resembled that of coniine; and it formed a hydrochloride.

In order to ascertain the physiological action of the volatile amide, approximately one centigram of its hydrochloride was dissolved in a very little water, absorbed in about one-half a gram of white bread, and given to a mouse. The mouse ate approximately one-half the mass of moistened bread greedily, then gradually stopped eating, and later showed no inclination to consume the remainder of the bread. In about one-half an hour, the animal became indolent, and remained so for about 20 minutes, then again behaved in a normal manner; these attacks of dullness and indolence returned several times. The mouse showed no inclination to drink, and now and then ate white bread, but refused to consume the remainder of the bread which had been treated with the hydrochloride. It urinated very frequently. Death occurred after 12 hours. Autopsy showed almost complete emptiness of the heart, marked extravasation of blood in the lungs, and slight quantities of blood in the brain. The stomach contained some viscous black material adhering to its walls. The intestines contained black feces alternating with gas; the gas was most abundant in the uppermost parts of the intestines. The bladder was completely filled. Björklund and Dragendorff decided that, while this single experiment did not permit a definite conclusion concerning the toxicological action of the volatile amide, yet it supported the view that this compound does not belong among the true poisonous alkaloids, and also indicated that the diuretic action of the rhizome may be due, at least partly, to the presence of this amide.

Both the rhizomes and the pitchers contained the same resin; but the chlorophyll content of the pitchers was included in the percent of resin reported for them.

The species of sugar present in the tissues was not determined.

The substance, similar to cinchona red, derived from the rhizomes, was soluble in alkalies with a red color.

The red pigment present in hydrochloric acid decoctions of the rhizome

was apparently derived from a chromogen occurring in the cambium layer, which dissolved readily in water, very difficultly in alcohol, and was converted into the pigment by cleavage by the acid. It was precipitated from its solution in hydrochloric acid as a violet pigment on addition of ammonia.

A proximate analysis of *Sarracenia purpurea* by Frohwein is included in a report on that plant by a committee of the New York County Medical Society.[26] Since the report deals with the use of the rhizome as a remedy for small-pox, rhizomes probably were the tissues analyzed. Frohwein reported the presence of gum, starch, vegetable albumin, tannin, resin, bitter principle with an acid reaction, extractive matter, traces of volatile oil, calcium, magnesium, potassium, sodium, iron, silica, phosphoric acid, sulphuric acid, and carbonic acid. "From this analysis it would seem that an alkaloid does not exist in the root of the *S. purpurea*, and it might be considered only a mild tonic, on account of the bitter principle which it contains."

Martin[27,28] found in the rhizomes of *Sarracenia purpurea* an alkaloid (sarracenine), a resin, a yellow coloring matter, extractives, and substances which form the skeleton of plants.

In order to obtain the alkaloid, sarracenine, the rhizomes were pulverized and made into a thin paste with distilled water acidified with sulphuric acid. This paste was kept in an oven until desiccation was complete. The powdery residue was extracted for four days in a flask with carbon disulphide, with agitation from time to time. The supernatant liquid was removed by filtration through linen under strong pressure. The woody residue on the filter contained the sarracenine; it was extracted with boiling distilled water in a capsule for one-half hour; the resulting solution was removed by filtration under strong pressure. The filtrate was again filtered, then evaporated on the water-bath to a syrupy consistency. This syrup was mixed with twice its volume of ethyl ether in a flask which was shaken vigorously at intervals for 1 or 2 days; the ether was then decanted, and permitted to evaporate spontaneously. The residue was dissolved in distilled water. The resulting solution was filtered if necessary, and was concentrated on the water-bath until the sarracenine sulphate crystallized. Pure sarracenine was obtained by mixing its sulphate with sodium bicarbonate, and isolating the free alkaloid by means of rectified alcohol.

Sarracenine is described as a white substance, soluble in alcohol and ether, and forming salts with acids. Its sulphate crystallizes in beautiful needles, and has a bitter taste which it imparts to its solutions.

Reference is at times made in the literature to an abstract of Martin's paper.[29]

Schmitt[30] carried out a series of experiments on dried *entire plants* of *Sarracenia purpurea*. They contained 11.43 percent of hygroscopic moisture (determined by drying at a temperature of 120° C.), and yielded 3.32 percent of a white ash. Analysis of the ash showed the presence of much potassium, also of calcium and the radicals of the following acids: sulphuric, phosphoric, silicic, and hydrochloric.

He speaks of the use of the decoction and the tincture of these plants in pharmacy, and of the occasional use of the powdered plant. The decoction was prepared by mixing 50 grams of the plant with 1 liter of water, and concentrating to a volume of 500 cc.; it had a brownish yellow color; the dose is given as one-half glass in 24 hours. The tincture was prepared by percolation or maceration of 1 part of the plant with 5 parts of 80 percent alcohol; it was green in color. Both the decoction and the tincture were acid to litmus; they were subjected to qualitative tests for the presence of various compounds.

As a result of his experiments, Schmitt reports the occurrence in the plant of the following substances: (1) Plant skeleton, (2) gums, (3) albuminous substances, (4) resins, (5) sarracenic acid, (6) sarraceno-tannic acid, (7) fats and waxes, (8) potassium and calcium salts of organic acids, (9) inorganic compounds, and (10) water.

Sarracenic acid is the name given to the coloring matter of the plant. It is described as almost, if not entirely, insoluble in water, ether, and petroleum ether, and dissolves most readily in alcohol. It is an acid with a bitter taste; and its compounds with the alkalies and alkaline earths have a characteristic yellow color. The yellow solution obtained by the action of the alkaline earths (lime or baryta) upon the tincture of the plant reacts with alum to form, as a precipitate, a beautiful yellow color-lake, while the filtrate from this precipitate is entirely colorless.

Sarraceno-tannic acid is described as a physiological tannin, belonging to the group of tannins which includes those occurring in coffee, catechu, and Peruvian bark.

When the dried entire plant was subjected to distillation with water, volatile products were not obtained.

Vines[31] made a study of glycerol extracts of the pitchers of *Sarracenia flava*. Some pitchers were extracted with glycerol without previous treatment

with acid; others were treated with 1 percent acetic acid for 24 hours in order to activate a zymogen (if present), then were extracted with glycerol. These glycerol extracts were then tested for the presence of a protease. A portion of the glycerol extract, a fragment of swollen fibrin, and 2 cc. of 0.2 percent hydrochloric acid were mixed and permitted to digest at a temperature of 40° C. The period of incubation is not stated, but probably was 6 or 8 hours. In this test, a partial or complete solution of the fibrin and a positive biuret reaction, yielded by the filtered digestion-fluid, were considered evidence of the presence of a protease in the glycerol extract. Vines found no evidence of the presence of a protease in the tissues of the pitchers of *Sarracenia flava*, although he was able to demonstrate the presence of a protease in the tissues of the pitchers of certain species of *Nepenthes* by means of this technic.

The glycerol extract of the pitchers of *Sarracenia flava* was found to contain sugar.

Hetét[32] examined "powder of the leaves," therefore dried pitchers, of *Sarracenia purpurea* from the isles of Saint Pierre and Miquelon. He found in them "an alkaline substance, whose properties are identical with those of veratrine. The crystallization is the same, in beautiful prisms and in octahedra of the orthorhombic system. It behaves in the same way with the principal neutral solvents; it gives the same reactions with the acids and the solutions used to distinguish the alkaloids, that is, particularly, the successive colorations with concentrated sulphuric acid, with sulphomolybdic acid, and especially hydrochloric acid and heat, which produce that beautiful, persistent, reddish violet coloration, quite peculiar to veratrine."

Hetét also found in the pitcher the amine previously discovered by Björklund and Dragendorff,[24,25] and "another alkaline substance, soluble in water."

Lambert[13] reports experiments, apparently made on fresh plants of *Sarracenia purpurea* on the isle of Saint Pierre and on the coast of Newfoundland. He studied sarracenic acid, the yellow coloring matter of the plant, which had been observed previously by Schmitt.[30] Lambert writes: "We have very easily recognized this coloring matter in the plant and have isolated it. We have ascertained that it has the singular property of being very soluble in alcohol, which it slightly colors. It suffices to add several drops of an acid (nitric acid seems to serve best) for the alcoholic solution to acquire a beautiful red color. On the other hand, several drops of any alkali

make the red color pass, after neutralization, to a dull green; and the least trace of an acid suffices to restore the color to its original red. This coloring matter, then, indeed behaves like a veritable acid and compares singularly in its reactions with litmic acid. The sensitivity of the coloration of this acid is such that it has appeared to us as capable of replacing litmus or phthalein in the titration of alkalies and of acids."

Lambert also tested the plants for the presence of a volatile base. A tiny fragment of the plant was treated with a dilute potash solution in a test-tube. When the tube and its contents were heated, the characteristic mouse-like odor of coniine was at once recognized.

The text does not state whether these experiments on the pigment and the volatile base were made on the pitcher, the rhizome, or the entire plant. However, attempts were made to isolate the base from the leaves (pitchers), but were fruitless; for the base was present in so small an amount that only the odor was obtained. It is suggested that this base acts as a lure for insects.

Gies[33] used fresh pitchers of *Sarracenia purpurea* in his experiments. The pitchers were thoroughly macerated in glycerol; and the resulting extract was used in tests for the presence of a protease. The glycerol extracts obtained from one set of plants showed moderate but distinct digestive action on fibrin at a temperature of 38° C. in the presence of "slight amounts of hydrochloric or oxalic acids"; the control experiments showed no digestion. The glycerol extracts from a second set of plants entirely lacked digestive action. Gies interprets these results: "In view of the negative results in the second series it is impossible at present to draw a satisfactory conclusion in this connection. It may be that the positive results in the first case were due to a bacterium specially favored by the medium furnished by the constituents of the glycerin extract, or to enzyme in unobserved diseased portions of the plants. Again, the negative results may have been due to a less favorable degree of acidity, or the secreting cells of the pitchers may have been in a 'resting condition' without either enzyme or zymogen." The two sets of plants came from different localities.

The diluted glycerol extract was practically colorless when neutral, crimson with acids, and green with alkalies. Further experiments showed that "Sarracenia purpurea contains a pigment which in concentrated glycerin extract has a reddish color, but which when diluted is practically colorless. At such dilution, when scarcely any color is to be seen, a drop of dilute acid

produces a bright pink throughout the whole fluid; alkali in minute amounts turns it green. The pink is converted to green by alkali, *vice versa* by acid. Even in crude glycerin extract the pigment appears to be very sensitive and may be used to advantage in titrimetric work. I have named the pigment alkaverdin, because of the beautiful green produced on treatment with alkali. . . . Excellent 'test papers' have been made with the pigment in glycerin extract. Ordinary filter paper dipped into the red, concentrated extract is colorless wet or dry. The dry paper turns a bright pink when dipped into acid, a deep green is produced when in contact with alkali." The solutions of alkaverdin had no special influence on the spectrum.

Both the aqueous and the saline extracts of the pitchers contained "an abundance of dextrorotatory, reducing and fermentable substances."

Meyer and Gies[34] found that alcohol extracted 3 coloring matters from macerated purple pitchers of *Sarracenia purpurea:* green chlorophyll, purplish red alkaverdin, and a brownish black substance. Water extracted only alkaverdin and the brownish black pigment from the pitchers. When the aqueous extract was evaporated almost to dryness *in vacuo* and was then poured into absolute alcohol, the brownish black pigment was precipitated. The color of this pigment was not influenced by either acid or alkali; it did not react with ferric chloride, and had no reducing power.

The alcoholic solution now contained all the alkaverdin; it was evaporated almost to dryness *in vacuo*. The residue had the color and consistency of dark molasses, a bitter taste, and an odor which was sugary and also resembled the characteristic odor of the macerated pitchers. It was insoluble in ether and chloroform, but soluble in water and alcohol, was free from the halogens, nitrogen, and sulphur, and contained a large amount of fermentable dextrorotatory carbohydrate which had a marked reducing action on Fehling solution and yielded a phenyl osazone resembling in crystal form that derived from glucose (dextrose). Removal of the sugar by fermentation had no apparent injurious action on the alkaverdin.

The aqueous solution of this molasses-like syrup had a reddish color, but became colorless when very dilute. Such a colorless solution became green on addition of a drop of 0.2 normal alkali, and returned to its colorless condition on addition of a drop of 0.2 normal acid. A drop or two of the acid in excess produced a pink color, quite different from the color of the syrup, and intensified

somewhat on standing. These changes in color were due to the presence of alkaverdin; and delicate test papers were made with that pigment.

Alkaverdin was not obtained in the crystalline state; it was found to have no influence on the spectrum. When its aqueous solution was warmed for some time on the water bath, the alkaverdin was converted into a brownish substance which still gave a green color with alkali, but did not yield a pink color with acid. Hydration with 2 percent sulphuric acid caused alkaverdin to lose completely its tinctorial properties.

Clark,[35] in the course of a research on plant oxidases, examined the "pitcher-plant leaves" of *Sarracenia Drummondi*, using an aqueous extract of the fresh pitchers for his experiments. The extract did not contain *oxygenase;* presence of *peroxidase* was doubtful; *catalase* was present; *chromogens*, which are oxidized to colored compounds by the natural oxidase of the plant, were not found.

For oxygenase (direct oxidase) seven reagents were used: tincture of guaiac, tincture of guaiac previously boiled with bone black to remove peroxides, α-naphthol, 1,4 phenylenediamine hydrochloride, phenolphthalein, phenol, and the indophenol reagent (α-naphthol plus 1,4 phenylenediamine hydrochloride in the presence of sodium carbonate). Hydrogen peroxide was added to each of these reagents in testing for peroxidase. Catalase was detected by its action in liberating molecular oxygen from a dilute solution of hydrogen peroxide.

A CHEMICAL STUDY OF THE NECTAR OF THE SARRACENIACEÆ

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D.

Either 25 or 50 pitchers of a given species were collected in the field while secreting nectar. They were cut from the plant near the base of the pitcher, and their tops were washed in succession with the same portion of distilled water (approximately 75 cc.). In this way an aqueous solution of the nectar was obtained free from pitcher liquor, prey, and plasma of the plant cells. The solution was filtered through filter paper to remove any insoluble particles, chiefly dust. The solution was immediately tested, by means of Benedict qualitative alkaline copper solution, for the presence of reducing sugar, and was then preserved by addition of sufficient trikresol to render the concentration of that bactericide 0.2 percent. Further tests were made in the laboratory at Philadelphia.

Separate series of tests were made on each of the following species: *Darlingtonia californica*, *Sarracenia minor*, *S. Sledgei*, *S. flava*, *S. Drummondii*, and *S. purpurea*.

The aqueous solution of the nectar of each of these species gave the following reactions:

(1) It reduced Benedict qualitative alkaline copper solution, showing the presence of a reducing sugar.

(2) It yielded the characteristic crystals of phenylglucosazone in the osazone test. This test was performed in two ways. The aqueous solution of the nectar was digested at the temperature of boiling water with phenylhydrazine hydrochloride and sodium acetate, then permitted to stand at the temperature of the room. Or the aqueous solution of the nectar was heated for one minute over a low flame after the addition of free phenylhydrazine and acetic acid; the solution was rendered almost, but not quite, neutral by addition of an approximately 14 percent aqueous solution of sodium hydroxide, was again heated for 1 minute, then permitted to stand at the temperature of the room. In either case crystals of an osazone were formed, and were identified as those of phenylglucosazone by microscopic examination.

(3) It gave a distinct crimson color in the Seliwanoff test. In this test, a portion of the aqueous solution of the nectar was mixed with its own volume of Seliwanoff reagent and then heated at the temperature of boiling water for exactly 20 minutes. The Seliwanoff reagent was an aqueous solution containing 0.05 percent resorcinol and approximately 12 percent hydrochloric acid. The development of the crimson color under the conditions of the test was evidence of the presence of a ketose sugar.

These reactions indicated that the nectar contained either fructose (levulose) or invert sugar.

The aqueous solutions of the nectar of *Darlingtonia californica* and *Sarracenia flava* were sufficiently concentrated to permit quantitative examination, both chemically and optically. In the *chemical* examination, the solution was titrated against Benedict quantitative alkaline copper solution to determine its reducing sugar content which was then calculated as glucose. This Benedict solution was standardized by titration with a freshly prepared solution of glucose of the highest purity. For the *optical* examination, the solution of the nectar was first clarified by addition of solid lead subacetate (Horne), thorough mixing, and filtration through a dry filter paper; this treatment removed all optically active compounds other than sugars. The filtrate was treated with solid neutral potassium oxalate to remove the excess of lead; and the resulting solution was filtered through a dry filter paper. This filtrate was used for the optical examination in a Peters saccharimeter.

The solution of the nectar of *Darlingtonia californica* was found, by the Benedict method, to contain 0.22 percent of reducing sugar, calculated as glucose. Examined in a standard tube, 200 mm. in length, it gave a reading of $-0.9°$ in the saccharimeter.

According to Browne,[69] the relative copper reducing values of the sugars in the volumetric method are:—Glucose 1.000, fructose 0.924, invert sugar 0.962. This author gives the following normal weights of sugars for saccharimeters with a Ventzke scale:—Fructose 18.592 grams, invert sugar 86.450 grams.

From the relative copper reducing values $\frac{\text{Glucose}}{\text{Frutcose}} = 0.924$ and $\frac{\text{Glucose}}{\text{Invert Sugar}} = 0.962$, it follows that the same copper reducing value is possessed by solutions containing 0.22 percent glucose, 0.24 percent fructose, and 0.23 percent invert sugar respectively. Therefore, the aqueous solution of the nectar contained either 0.24 percent fructose or 0.23 percent invert sugar.

From the normal weights, it follows that a solution producing a rotation of −0.9 saccharimeter degree may contain 0.009 times 18.592 or 0.17 gram of fructose in 100 cc., *i. e.*, 0.17 percent fructose. Or it may contain 0.009 times 86.450 or 0.78 gram of invert sugar in 100 cc., *i. e.*, 0.78 percent invert sugar.

Since the values for fructose, obtained by copper reduction (0.24 percent) and by the optical method (0.17 percent), show a far closer agreement with each other than do those for invert sugar (0.23 percent by copper reduction and 0.78 percent by the optical method), it follows that the reducing sugar present in the nectar of *Darlingtonia californica* is fructose (*d*−fructose, levulose) and not invert sugar.

The solution of the nectar of *Sarracenia flava* was found, by the Benedict method, to contain 0.17 percent of reducing sugar, calculated as glucose. Examined in a tube 400 mm. in length, it gave a reading of −1.4° in the saccharimeter; this corresponded to a reading of −0.7° for a standard tube 200 mm. in length.

From the relative copper reducing values, it follows that the same reducing value is possessed by solutions containing 0.17 percent glucose, 0.18 percent fructose, and 0.18 percent invert sugar, respectively.

From the normal weights, it follows that a solution producing a rotation of −0.7 saccharimeter degree may contain 0.13 percent fructose or 0.61 percent invert sugar.

Since the values for fructose, obtained by copper reduction (0.18 percent) and by the optical method (0.13 percent), show a far closer agreement with each other than do those for invert sugar (0.18 percent by copper reduction and 0.61 percent by the optical method), it may be concluded that in *Sarracenia flava* also, the reducing sugar present in the nectar is fructose and not invert sugar.

OBSERVATIONS ON THE PITCHER LIQUOR OF THE SARRACENIACEÆ

By FRANK MORTON JONES, F.E.S., and
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Volume of the Normal Pitcher Secretion

The pitchers of all North American *Sarraceniaceæ* grow to a considerable size and even approach their full size before the orifice of the pitcher opens. These hermetically sealed or closed pitchers normally contain a larger or smaller volume of clear fluid which is transparent and colorless, though sometimes rendered slightly milky by suspended particles. The volume of the fluid or pitcher liquor is much greater in large vigorous pitchers than in small stunted ones in which the liquor may be almost entirely absent. In *Sarracenia purpurea* and *S. psittacina*, the quantity of pitcher liquor is very small; even in the larger pitchers, it usually appears only as a studding of minute perspiration-like drops on the lower wall and rarely collects in the bottom of the cavity. In all the other species, the pitcher liquor occurs in sufficient quantity to be drawn off with a pipette, and may fill the narrow tube to a height of several inches in vigorous pitchers.

After opening, the pitchers of *Sarracenia purpurea* usually become filled, entirely or partly, with rain water. Certain species, especially *Sarracenia purpurea* and *S. psittacina*, may be partially or completely submerged in time of flood as a result of their shape, size, and habitat. Beating rains may enter, in greater or less degree, the pitchers of the remaining species of *Sarracenia*. The level of the liquid contents of the pitcher, thus augmented, may be reduced again by evaporation or absorption. In dry weather, and especially when the soil about the roots becomes dry, the pitchers may be found to have lost much or all of their liquid contents. Under these varying circumstances, the contents of an open pitcher may consist principally of a mass of insect remains ranging from dry and friable to moist and broth-like, or of insect cadavers bathed in a liquid which may range from clear to markedly turbid, or of insect captures floating in an excess of liquid (rain water or flood water) which, necessarily, highly dilutes the secretion of the pitcher.

The volume of the pitcher liquor, reported in Table I for *Darlingtonia*

californica, *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii* are based on measurements of the volume present in normal, full-sized pitchers—closed, plugged, or open—of these species growing in favorable localities at the height of their season and not materially affected by external conditions. The volumes recorded represent the amount of secreted liquid present in the pitchers at the time of examination. *Closed* pitchers were used, as nearly as possible, at their point of opening. *Plugged* pitchers, in age and development, were open and active but free from insect captures. The top of a closed pitcher, which was about to open, was folded over; a piece of paper bearing the date was included in the fold; and one or more pins were passed through the doubled pitcher and paper, effectually sealing the top. The pitcher did not wither, but continued to grow and to expand its top even above the fold; it was permitted to remain in this condition for 1 week in the case of the Sarracenias and for as long as 1 month in the case of *Darlingtonia californica*. *Open* pitchers were fully mature, and actively engaged in the capture of insects.

Determination of the volume.—The upper portion of the pitcher was removed with scissors until the pitcher liquor was within reach of a plain pipette, the lower portion of which was of fine bore. As each portion of the liquor was removed by means of the pipette, another section of the pitcher was cut off. This procedure was repeated until the entire liquid contents of the pitcher had been collected. The total liquid contents of a definite number of pitchers were collected in a stoppered vial by transfer from the pipette. The total volume obtained from the lot was measured in a narrow graduated cylinder; and the average volume per pitcher was calculated.

Not many pitchers of *Sarracenia rubra* were obtainable. The pitchers of *Sarracenia minor* were of a size far below the recorded maximum for this species. Therefore quantitative measurements of the volume of liquor present in the pitchers of these two species have not been reported. No records are included for *Sarracenia psittacina* since the pitchers of this species do not secrete liquor in measurable quantity during their seasonal history. The liquid ordinarily present in open pitchers of *Sarracenia purpurea* is the secretion of the pitcher highly diluted by rain water, for the shape of the pitcher of this species readily permits the entrance of falling rain; therefore no measurements were made of the volume of liquor present in such pitchers. The amount of liquor present in closed pitchers of this species is so slight that its volume is not measureable.

Observations and measurements made on many individual pitchers, as well as the general results presented in Table I, indicate that the pitcher liquor progressively increases in volume as the pitchers develop. A further marked increase in the volume of the pitcher liquor occurs after the lid and lips have opened, and a still further increase is usually apparent after the capture of prey.

On account of the shape of the pitchers and the habitat of the plant, the volume of the pitcher liquor in *Darlingtonia californica* is less influenced by external conditions than in the Sarracenias. The progressive increase in the volume of the pitcher liquor is shown by the following data. As reported in Table I, 82 *plugged* pitchers, studied in 4 lots during June and July, contained, on the average, 2.50 cc. of liquor per pitcher. A single lot of 25 pitchers, plugged in June and studied during the following September, in the same bog as the other lots, contained, on the average, 3.38 cc. of liquor per pitcher. As reported in Table I, 161 *open* pitchers, examined in 5 lots during June and

TABLE I.—AVERAGE VOLUME OF THE LIQUOR IN THE PITCHERS OF CERTAIN SARRACENIACEÆ.

	Closed Pitchers			*Plugged Pitchers*			*Open Pitchers*		
Genus and Species.	*Number of lots measured.*	*Total number of pitchers.*	*Average volume per pitcher, cc.*	*Number of lots measured.*	*Total number of pitchers.*	*Average volume per pitcher, cc.*	*Number of lots measured.*	*Total number of pitchers.*	*Average volume per pitcher, cc.*
Darlingtonia californica	10	353	1.08	4	82	2.50	5	161	2.80
Sarracenia Sledgei	4	325	0.25	2	31	0.38	3	74	0.38
Sarracenia flava	6	566	0.66	1	8	1.30	4	160	1.50
Sarracenia Drummondii	4	230	0.44	1	20	0.66	4	138	0.58

July, contained, on the average, 2.80 cc. of liquor per pitcher. A single lot of 25 open pitchers, examined during the following September in the same bog as the other lots, contained, on the average, 4.04 cc. of liquor per pitcher.

The volume of pitcher liquor varies widely in any given species with the size, vigor, and degree of development of the pitcher. Thus in the 10 lots of *closed* pitchers of *Darlingtonia californica*, which were strictly comparable with each other, the maximum average volume of pitcher liquor for any lot was 1.60 cc. per pitcher, while the general average for all 10 lots was 1.08 cc. per pitcher. Also in the 5 lots of *open* pitchers of this species, likewise strictly comparable with each other, the maximum average volume of pitcher liquor for any lot was 3.61 cc. per pitcher, while the general average for all 5 lots was

2.80 cc. per pitcher. Individual pitchers of unusual size or vigor frequently contained pitcher liquor far in excess of the maximum average volumes just reported.

Reaction of the Pitcher Liquor

The reaction of the pitcher liquor to litmus was determined for the species mentioned below, using liquor from individual pitchers and composite samples of liquor from many pitchers. The mode of collection of the pitcher liquor, described above, yielded a liquid uncontaminated by the plasma of the pitcher tissues. Blue and red litmus paper (Squibb) was used; this reagent is sensitive to 0.004 normal acid and alkali respectively.

TABLE II.—REACTION OF THE PITCHER LIQUOR TO LITMUS

Genus and Species.	*Closed Pitchers.*	*Plugged Pitchers.*	*Open Pitchers.*
Darlingtonia californica	neutral	neutral	neutral
Sarracenia minor	neutral		neutral to faintly acid
Sarracenia Sledgei	acid	usually neutral	neutral
Sarracenia flava	acid		neutral to faintly acid
Sarracenia Drummondii	markedly acid	usually neutral	neutral
Sarracenia rubra	slightly acid		

The reaction of the pitcher liquor showed variations in the individual plugged pitchers of two species. In the case of *Sarracenia Sledgei*, the liquor was neutral in 20 pitchers, acid in 3 pitchers, and faintly alkaline in 2 pitchers. In the case of *Sarracenia Drummondii*, the liquor was neutral in 13 pitchers, acid in 5 pitchers, and faintly alkaline in 2 pitchers.

The change from an acid to a neutral reaction, which occurs in the pitcher liquor of several species upon the opening of the pitchers, does not depend upon or result from the capture of prey, for the same change occurs in plugged pitchers from which insects are carefully excluded.

Mechanical Stimulation

In a series of experiments on mechanical stimulation of the pitchers, efforts were made to simulate the struggles of entrapped insects by dangling strings of glass beads, gentle scratching with elastic wire brushes, etc. These experiments were made on *Darlingtonia californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*, using pitchers of all ages, closed, just open, and open active pitchers both with and without prey. The period and degree of stimulation and also the elapsed time between stimulation and withdrawal of the pitcher contents were varied. However, no measurable effect upon the volume of the pitcher liquor was detected in any of the experiments.

Food Stimulation

Reference has been made to the work of Mrs. Austin[7] on the response of *Darlingtonia californica* to food stimulation. An account of many additional experiments on such stimulation is given by her in her unpublished letters and manuscript journal, which are preserved among the Canby Papers deposited in the library of the Society of Natural History of Delaware at Wilmington.

In our experiments, use has been made of milk, beef broth, meat, other foods of animal origin, and inorganic salts.

Milk.—Fresh skim milk was diluted with its own volume of water, sterilized by heat, and permitted to cool to the temperature of the atmosphere. Measured volumes of this milk were introduced into the pitchers by pouring from a narrow graduated cylinder. With closed pitchers of the Sarracenias, with plugged pitchers of this genus, and with all three types of pitchers of *Darlingtonia californica*, the top of the pitcher was cut off just beneath the lips or pitcher rim; the milk was introduced; the uppermost portion of the pitcher was folded over; a piece of paper bearing a memorandum of the experiment was included in the fold; one or more pins were passed through the doubled pitcher and paper, effectually sealing the pitcher cavity. With open pitchers of the Sarracenias, the milk was introduced through the open mouth of the pitcher which was then sealed in the manner just described.

At the end of a given period of time, the volume of the liquid contents of the pitcher was determined in the manner already described. When more than one pitcher was used in an experiment, the average volume of liquid contents per pitcher was determined at the end of the period of stimulation. The "Percentage increase above normal liquid contents" was calculated as follows. From the average volume of liquid contents per pitcher at the end of the period of stimulation was subtracted the sum of the volume of milk introduced plus the average volume of the liquid contents per pitcher in pitchers of the same type and species, using the values given in Table I. The remainder, which represented the volume of liquid secreted by the pitcher in response to stimulation, was divided by the average volume of the liquid contents per pitcher in pitchers of the same type and species as recorded in Table I. The quotient was multiplied by 100. The final arithmetical result expressed the volume of the liquid produced by stimulation as percent of the volume of liquid usually present in pitchers of the type and species used in the experiment. The average values in Table I were used, since the determinations

reported in that table and the stimulation experiments were made at the same time and place for a given species.

TABLE III.—RESPONSE OF THE PITCHERS OF CERTAIN *SARRACENIACEÆ* TO STIMULATION BY MILK.

Genus and Species	*Number of pitchers.*	*Type of pitcher.*	*Volume of milk introduced into each pitcher.*	*Period of stimulation. Days.*	*Percentage increase above normal liquid contents.*
Darlingtonia californica	7	closed	1	1	20
" "	12	"	3	1	86
" "	8	"	3	1	38
" "	6	"	3	3	200
" "	4	"	3	6	401
" "	1	"	12	6	1242
" "	7	plugged	1	1	37
" "	6	"	1	3	63
" "	3	"	15	6	532
" "	1	"	5	7	476
" "	4	"	12	7	446
" "	6	open	3	3	57
" "	1	"	15	5	292
" "	2	"	30	5	430
" "	6	"	3	6	119
" "	1	"	15	6	328
" "	1	"	45	6	435
" "	1	"	10	10	471
" "	1	"	30	10	578
Sarracenia Sledgei	4	plugged	5	5	952
" *flava*	4	closed	10	4	1096
" "	3	open	10	4	268
" *Drummondii*	4	plugged	5	5	506

The results, which have been summarized in Table III, demonstrate that the pitchers of the species studied poured out additional pitcher liquor in response to their stimulation by the introduced milk. The volume of additional liquor thus secreted depended in part on the volume of milk introduced, and in part on the period of time during which the stimulus was permitted to act.

When 30 cc. or more of milk were introduced into a pitcher, it became necessary to support the pitcher.

In all the experiments on food stimulation, injury to the pitcher was observed in but one experiment, which has not been included in those tabulated. An open pitcher of *Darlingtonia californica* received 30 cc. of milk; 10 days later, the pitcher was characterized by a brown discoloration and shrunken areas indicative of serious injury. A similar condition has been noted in the field in pitchers of the Sarracenias which contained a very unusual bulk of insect captures.

Beef broth.—Raw lean beef was cut into fine strips, covered with water,

and permitted to simmer for one hour. The resulting broth was strained and cooled. The broth was used in stimulation experiments; the technic was exactly the same as when milk was used.

In all the experiments on *Darlingtonia californica*, the period of stimulation was 5 days. Each of 4 closed pitchers received 5 cc. of the broth; the stimulation produced an increase above the normal average volume of the liquid contents per pitcher of 387 percent. Each of 5 plugged pitchers received 10 cc. of broth; the resulting increase above the normal liquid contents was 308 percent. Fifteen cc. of the broth were introduced into an open pitcher; an increase above the normal liquid contents of 346 percent resulted.

In each of the experiments on *Sarracenia flava*, 9 pitchers were used; 10 cc. of broth were introduced into each pitcher; and the period of stimulation was 4 days. Closed pitchers showed an average increase above the normal liquid contents of 1787 percent, and a maximum increase of 2445 percent. Open pitchers showed an average increase of 683 percent, and a maximum increase of 1060 percent.

Meat.—Both raw beef and beef cooked by boiling were used in separate series of experiments on *Darlingtonia californica*. From 2 to 8 cubes (length of edge one-eight inch) of meat were dropped into each pitcher; the period of stimulation ranged between 5 and 7 days; details concerning the preparation of the pitchers and the measurement of the liquid contents have been given under the experiments with milk. An increase in the average volume of the pitcher contents was not noted when *cooked* beef was introduced into either plugged or open pitchers. The increase observed after the introduction of *raw* beef ranged from 48 percent in one lot of closed pitchers to 157 in one lot of open pitchers; intermediate values were noted in other pitchers of all three types.

The failure of the meat, raw or cooked, to produce a large increase in the volume of pitcher liquor, such as was produced by milk or by beef broth, is probably due to the fact that the solid meat exerted any stimulating action only on the small area of the pitcher lining with which it was in contact. The liquid foods occupied a larger volume and, therefore stimulated the far larger area of the pitcher lining with which they were in intimate contact. The bulk of the meat introduced did not equal that of the insect captures of many open pitchers; and an increase of the liquor to a volume far greater than that present in open pitchers was not to be expected.

Other foods of animal origin.—Experiments, similar to those with meat, were made on *Darlingtonia californica*, using raw egg white, cubes of the white of hard-boiled eggs, cheddar cheese, casein (Hammarsten), and fibrin. No definite response to the introduction of these substances was observed, probably for the same reasons as with meat.

Inorganic salts.—The following salts were used in aqueous solution: ferrous sulphate, ferric ammonium sulphate plus sodium acetate, potassium ferrocyanide, and potassium ferricyanide.

Five cc. of a 2 percent solution of ferrous sulphate were introduced into several pitchers of each of the following species: *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*. The pitchers were not injured at the end of several days, and an increase occurred in the volume of the pitcher liquor; in pitchers of *S. flava*, the liquor had increased to 4 times its normal volume.

A solution was prepared containing 2 grams of ferric ammonium sulphate (iron ammonium alum) and 2 grams of sodium acetate in each 100 cc.; the sodium acetate was added for its buffer effect, *i. e.*, to lessen the acidity otherwise imparted to the solution by the alum. This solution was introduced into pitchers of the three species mentioned above, 5 cc. to each pitcher. Several days later, the pitchers were obviously dying.

Potassium ferrocyanide and potassium ferricyanide were used in separate series of experiments on both closed and open pitchers of *Sarracenia flava*. Five cc. of a dilute solution of one of these salts were introduced into each pitcher; 4 days later the volume of the liquid contents of the pitcher was measured. Both salts produced an abundant secretion of pitcher liquor; this phenomenon was more marked in the case of potassium ferrocyanide. The increased secretion in one closed pitcher was almost 21 times the average normal secretion.

Response to Introduced Acid and Alkali

In the experiments on stimulation with milk, the pitcher contents frequently became acid to litmus. In order to ascertain whether the observed increase in the volume of the pitcher secretion was due to milk *per se* or to the acidity developed, the following studies were made with dilute acids. The response to the acids led to similar experiments with dilute alkali. The technic was that used in the experiments with milk. Except in two experiments, the reagent was permitted to remain in the pitcher for 5 days; in the experiments with single pitchers of *Sarracenia Sledgei* reported in Table IV, the acid re-

mained in the pitchers for 8 days. The reagents used were hydrochloric acid (symbol HCl, either 0.05 or 0.2 percent aqueous solution), acetic acid (abbreviated AcOH, either 0.05 or 0.1 percent aqueous solution), and sodium hydroxide (symbol NaOH, either 0.02 or 0.05 percent aqueous solution).

The following conclusions are deduced from a study of the data reported in Table IV:

TABLE IV.—RESPONSE OF THE PITCHERS OF CERTAIN *SARRACENIACEÆ* TO INTRODUCED ACID AND ALKALI

Genus and species.	*Number of pitchers.*	*Type of pitcher.*	*Reagent.*	*Volume of reagent introduced into each pitcher, cc.*	*Average volume of pitcher contents at end of experiment, cc.*	*Reaction of pitcher contents to litmus at end of experiment.*
Darlingtonia californica	15	Plugged	0.2% HCl	3.0	6.00	Neutral
Darlingtonia californica	6	Open	0.05% HCl	3.0	5.40	Neutral
Darlingtonia californica	1	"	0.05% HCl	10.0	11.00	Neutral
Darlingtonia californica	7	"	0.2% HCl	3.0	5.65	Neutral
Darlingtonia californica	6	"	0.2% HCl	10.0	*	Acid
Darlingtonia californica	2	"	0.2% HCl	30.0	*	Acid
Sarracenia Sledgei	2	"	0.05% HCl	5.0	2.20	†
" "	1	"	0.05% HCl	5.0	<0.50	Neutral
" *flava*	1	Closed	0.05% HCl	5.0	3.60	Faintly acid
" "	2	Open	0.05% HCl	5.0	5.00	Faintly acid
" *Drummondii*	5	"	0.05% HCl	5.0	3.55	Neutral
Darlingtonia californica	17	Plugged	0.1% AcOH	3.0	6.15	Neutral
Darlingtonia californica	1	Open	0.1% AcOH	3.0	6.00	Neutral
Sarracenia Sledgei	2	"	0.05% AcOH	5.0	2.10	Neutral
" "	1	"	0.05% AcOH	5.0	<0.50	Neutral
" *flava*	1	Closed	0.05% AcOH	5.0	2.80	Neutral
" "	3	Open	0.05% AcOH	5.0	2.67	Acid
" *Drummondii*	4	"	0.05% AcOH	5.0	1.20	Neutral
Darlingtonia californica	15	Plugged	0.02% NaOH	3.0	6.07	Neutral
Darlingtonia californica	4	Open	0.02% NaOH	3.0	6.80	Neutral
Sarracenia Sledgei	2	"	0.05% NaOH	5.0	4.50	Neutral
" *flava*	1	Closed	0.05% NaOH	5.0	4.00	Neutral
" "	1	Open	0.05% NaOH	5.0	2.40	Neutral
" *Drummondii*	3	"	0.05% NaOH	5.0	3.60	Neutral

Darlingtonia californica showed no marked trend to either increase or decrease the volume of its pitcher contents as a result of the introduction of dilute acid or dilute alkali.

* Pitchers injured, large areas brown and shrivelled.

† Contents of one pitcher faintly acid, of other pitcher neutral.

Almost invariably the volume of the liquid withdrawn from the pitchers of the Sarracenias was less than the volume of the introduced reagent. This shrinkage in volume was even greater than that shown by a comparison of the volume of reagent introduced with the average volume of the pitcher contents at end of experiment as given in Table IV, for the normal volume of pitcher liquor, which has already been reported in Table I, has not been included in Table IV. Therefore, evidence of absorption in the genus *Sarracenia* is unmistakable.

The reaction of the withdrawn pitcher contents was determined by means of litmus paper. The reaction, recorded in Table IV, should be compared with the reaction of the liquor of similar pitchers of the same species as reported in Table II. The pitcher contents of *Darlingtonia californica* returned to their normal neutral reaction within 5 days after the introduction of either dilute acid or dilute alkali. The contents of open pitchers of both *Sarracenia Sledgei* and *S. Drummondii* finally returned to their normal neutral reaction after introduction of any one of the three reagents. *Sarracenia flava* likewise showed a marked tendency for a return of its pitcher contents to their normal reaction within a few days after the introduction of either dilute acid or dilute alkali. This return of the pitcher contents to their normal reaction recalls the behavior of the human stomach under somewhat similar conditions as shown by the researches of Spencer, Meyer, Rehfuss, and Hawk.[70]

Action of the Pitcher Liquor of the Sarraceniaceæ upon Living Insects

Even prior to the opening of the pitcher, the pitcher liquor of certain species possesses the property of quickly rendering helpless those insects which come into contact with it. Reference has already been made to the work of Mellichamp[4, 16] and Watson,[17] who studied the action of the pitcher liquor of *Sarracenia variolaris* (*S. minor*) upon insects. In the present research similar studies have been made, using the pitcher liquor of four other species of the *Sarraceniaceæ*. Unless otherwise stated, the pitcher liquor was collected on the same day that its action on insects was tested.

A typical experiment may be described. A location was chosen adjacent to a populous crater-shaped nest of a large brown ant abundant at Summerville, South Carolina. Wide mouth vials were used as containers. Pitcher liquor from *Sarracenia flava* was introduced into 10 vials, 2 cc. to the vial; 5

vials received liquor from closed pitchers and 5 vials liquor from open pitchers. Water was placed in another set of 10 vials, 2 cc. to the vial, in order to serve as a control experiment. The vials were of uniform size, and were arranged in suitable order for convenient observation. Worker ants were picked from the nest gently with the fingers; one ant was dropped into each vial, and the time recorded. Note was made of the time at which each ant ceased its struggles to escape, assumed a rigid position, and became incapable of motion even on jarring the vial. The elapsed time between the introduction of the ants and the cessation of their struggles ranged from 0.5 to 3.0 minutes with an average of 1.4 minutes in the experiments with liquor from closed pitchers, and from 1.5 to 2.0 minutes with an average of 1.6 minutes in the experiments with liquor from open pitchers. In the control experiments with water, 5 of the 10 ants ceased motion, the elapsed time ranging from 3.0 to 9.5 minutes, while the other 5 ants were still active at the end of 30 minutes.

Liquor from open pitchers of *Sarracenia flava* was collected at De Funiak Springs, Florida, in May. It was kept at room temperature without addition of a preservative, and was tested at Wilmington, Delaware, 49 days later, using the above technic. Large black ants of the genus *Campanotus* served as the experimental animals, 6 ants in the experiment proper and 6 ants in the control experiment. The average elapsed time to cessation of motion was 20 *seconds* in the experiment proper. In the control experiment, 3 of the ants ceased motion in from 5.25 to 7.45 *minutes*, while the other 3 ants were still active at the end of 46 *minutes*.

Experiments were made with the pitcher liquor of *Sarracenia Sledgei* at Mobile, Alabama, using large black ants of the genus *Campanotus*. The average elapsed time to cessation of motion was 48 seconds when 5 ants were introduced into liquor from open pitchers. Five ants were used in the control experiments, 1 ceased motion at the end of 180 seconds, the others were still active at the end of 300 seconds.

Experiments were also made on liquor from open pitchers of *Sarracenia Drummondii* at De Funiak Springs, Florida, using large brown ants. Five ants were introduced into the pitcher liquor and ceased motion in from 20 to 30 seconds; 5 ants which were introduced into distilled water, retained their motility for many minutes, and were finally permitted to escape at the conclusion of the experiment.

Typical experiments with the pitcher liquor from *Sarracenia Sledgei*,

S. flava, and *S. Drummondii* have been reported above. Similar results, concerning the action of the pitcher liquor on insects, have been obtained in other experiments—not reported in detail—on liquor from closed pitchers and from open pitchers of these species. The action of the pitcher liquor of *Darlingtonia californica* upon insects is described in a subsequent section.

Death of the ants was not coincident with their cessation of motion. Thus in the second experiment reported above (that made on black ants at Wilmington, Delaware), 6 ants ceased motion, on the average 20 seconds after their introduction into the pitcher liquor. Fifteen minutes after motion had ceased, they were removed from the pitcher liquor and placed on bibulous paper. They showed evidences of motion, on the average, 6 minutes, 22 seconds later, and were able to run away, on the average, 9 minutes after their removal from the liquor. The ants from other experiments behaved in a similar manner. However, permanent cessation of motion of the captured insects must inevitably lead to their death within the pitchers.

Insects sank in the pitcher liquor of the Sarracenias more frequently and more promptly than in water in the control experiments. This phenomenon suggested that the pitcher liquor has a surface tension less than that of water, and acts upon the insects by virtue of this property. Chemical, physical, and physiological tests were made in this connection.

Chemical, Physical, and Physiological Examination of the Pitcher Liquor

These tests were made with pitcher liquor to which no bactericide had been added.

Test for saponin.—Since saponins lower the surface tension of water when dissolved in that solvent, the hemolysis test for saponins was applied to samples of the pitcher liquor in order to ascertain if compounds of that group were present. The finger was pricked and blood was permitted to fall into 0.5 cc. of a 0.1 percent solution of sodium citrate in physiological (0.85 percent) solution of sodium chloride, until the volume of the latter had doubled. Equal volumes of the resulting suspension of human erythrocytes and the pitcher liquor were mixed on a glass slide, covered with a cover slip, and then viewed through a microscope. The erythrocytes remained intact at the end of 30 minutes; therefore, hemolysis did not occur, and saponins were not present in the pitcher liquor. This test was applied to samples of liquor from closed pitchers of *Darlingtonia californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drum-*

mondii, and from open pitchers of *S. flava* and *S. purpurea*. With all 6 samples, the test for a saponin was invariably negative.

The *physical tests* were both qualitative and quantitative. An oiled needle was used for a qualitative measurement of the surface tension. A needle 1.25 inches in length was oiled with olive oil. It readily floated on the surface of water for its entire length, and was not readily sunken by jarring the sides of the containing vessel. The oiled needle was also placed on the surface of the liquor from open pitchers of *Sarracenia flava* which was used in the experiments made on ants at Wilmington, Delaware, and reported above. The needle was floated on the surface of the pitcher liquor with difficulty. The needle then floated with both ends submerged, and sank very readily when the sides of the containing vessel were gently jarred. The same oiled needle was transferred, by means of dissecting forceps, 20 times in succession from the water to the pitcher liquor, then back to the water. *Throughout the entire series of transfers*, the needle was readily floated on the water and was not sunken in that medium by a heavy jarring of the table; on the other hand, the needle was floated on the pitcher liquor with difficulty and readily sank in the liquor on the slightest jarring of the table.

Quantitative measurement of the surface tension of the liquor from closed pitchers of *Sarracenia flava* was made by means of the du Noüy surface tension apparatus,[71] as manufactured by the Central Scientific Company.[72] The measurements were made at a temperature of 25° C. The pitcher liquor was found to have a surface tension of 66.4 dynes per cm., while a sample of distilled water, tested at the same time, was found to have a surface tension of 75.0 dynes per cm. Hence the surface tension of the pitcher liquor was considerably lower than that of water.

Physiological tests.—The action of liquor from closed pitchers of *Sarracenia flava* was tested upon frogs and a guinea-pig.

The pitcher liquor was injected into the anterior lymph sac of *frogs;* 5 animals were used; the dose per gram of body weight ranged from 0.0035 to 0.05 cc., and the actual dose from 0.12 to 0.49 cc. The frogs were observed at intervals for 9 hours, and remained alive and active. The next day, two of the frogs were pithed. In both of them, the lymph sac showed no evidence of irritation; however, it was practically free from mucus which is normally present. Possibly the mucus had been digested by the proteolytic enzyme of the pitcher liquor.

One cc. of the pitcher liquor was injected subcutaneously into a *guinea-pig* weighing 527 grams; no evil effects were noted. Three days later, 3 cc. of the same sample of pitcher liquor were administered subcutaneously to the same guinea-pig without the production of any evil effects. Five days after the second injection the guinea-pig showed some adhesions in the region of the injections but was otherwise uninjured.

Action of the Pitcher Liquor of *Darlingtonia californica* Upon Living Insects

The action of the liquor from both closed and open pitchers of *Darlingtonia californica* was tested on ants, locusts (short-horned grasshoppers, family *Acrididæ*), and bumble bees (genus *Bombus*). These experiments were made in Plumas County, California.

In one experiment, 25 vials were prepared, and 3 cc. of liquor from closed pitchers were introduced into each vial. For controls, 3 cc. of water were introduced into each of another group of 25 vials. A large ant was dropped into each vial. The number of ants still active and struggling to escape was:– At the end of 1 minute, pitcher liquor 18, water 17; at the end of 5 minutes, pitcher liquor 11, water 8; at the end of 10 minutes, pitcher liquor 8, water 8; at the end of 30 minutes, pitcher liquor, 6, water 8. These results showed practically no difference between the pitcher liquor and the water with respect to their action on ants.

Similar experiments were made with bumble bees and with locusts, using liquor from closed and from open pitchers separately; the period of observation extended over several hours in each experiment. The results showed that the action of the pitcher liquor on these insects with respect to motility did not differ materially from that of water.

None of the insects—ants, bees, or locusts—sank in the pitcher liquor more frequently or more promptly than in water.

THE ENZYMES OF THE PITCHER LIQUOR OF THE SARRACENIACEÆ

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Protease

A series of experiments on the occurrence of a protease in the pitcher liquor of *Sarracenia flava* was described at the general meeting of the American Philosophical Society in 1917.[18] These experiments have been continued and extended to include the other North American representatives of the family. Each species has been studied separately. Tests have been made on liquor from closed pitchers and on filtered liquor from open pitchers. In the field experiments, carmine fibrin was used as the substrate. This substrate and others were used in laboratory experiments. As a rule, the test for the presence of a protease by means of carmine fibrin was made in an acid medium containing 0.2 percent hydrochloric acid, also in an alkaline medium containing 0.5 percent sodium carbonate; frequently it was also carried out without the addition of either acid or alkali; in every case, trikresol (0.2 percent) was used as a bactericide. Two reagents were prepared; one contained 2.2 percent hydrochloric acid and 2.2 percent trikresol; the other contained 5.5 percent sodium carbonate and 2.2 percent trikresol; by addition of 1 volume of a reagent to 10 volumes of pitcher liquor, the proper degree of acidity or alkalinity was imparted to the reaction mixture. Control or blank tests were carried out, using pitcher liquor which had been boiled, then cooled to the temperature of the atmosphere prior to addition of the reagents. The temperature of digestion was that of the atmosphere. Each separate test was usually made in duplicate, however, at times, in triplicate or even in quadruplicate. Unless otherwise stated, each test was made on a composite sample of liquor drawn from a number of pitchers.

With four species—*Darlingtonia californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*—edestan and casein were used as substrates in laboratory tests for the presence of a protease in the pitcher liquor. In these tests, the temperature of digestion was 37.5° C. The pitcher liquor had been preserved by addition on one-tenth its volume of a 2.2 percent aqueous solution of

trikresol; therefore 1.1 cc. of preserved liquor equalled 1.0 cc. of liquor as drawn from the pitchers.

In the experiments with *edestan*, a solution of that substrate was prepared by solution of 0.1 gram of edestin in 100 cc. of 0.1 percent hydrochloric acid by heating to boiling, then cooling to room temperature. In each experiment 1.1 cc. of preserved pitcher liquor were mixed with 2.0 cc. of edestan solution. After incubation for 1.5 hours, 0.5 cc. of a saturated aqueous solution of sodium chloride was added to the reaction mixture. If digestion of the substrate had not occurred, a cloudy precipitate of edestan formed; if partial digestion had occurred, a faint cloud formed; if the edestan had been completely digested, the solution remained perfectly clear. The controls in these experiments always yielded a cloudy precipitate.

In the experiments with *casein*, a freshly prepared solution of that substrate was used. A beaker, containing 0.1 gram of purified casein (caseinogen), 5 cc. of 0.1 normal sodium hydroxide solution and 25 cc. of distilled water, was placed on a wire gauze and heated until boiling occurred and the casein dissolved. The beaker and its contents were quickly cooled to room temperature. The solution was rendered neutral by addition of 0.1 normal hydrochloric acid, and was diluted to a total volume of 100 cc. In each experiment, 1.1 cc. of preserved pitcher liquor were mixed with 2 cc. of the substrate solution. The mixture was incubated at a temperature of 37.5° C., usually for 2 hours. Then 6 drops of a solution of acetic acid in dilute alcohol (1 part by volume of glacial acetic acid, 49 parts of distilled water and 50 parts of 95 percent alcohol) were added. If digestion of the substrate had not occurred, a cloudy precipitate of casein formed; if partial digestion had occurred, a faint cloud formed; if complete digestion had occurred the solution remained perfectly clear. The controls always yielded a cloudy precipitate. Whenever the pitcher liquor was so acid in reaction that a precipitate immediately formed on mixing it with the substrate solution, then the experiment was repeated using pitcher liquor which had previously been rendered neutral to litmus by addition of an aqueous solution of sodium carbonate.

Coagulated egg white was used as the substrate in certain experiments on the liquor from closed pitchers of *Sarracenia flava*, *S. Drummondii* and *S. Sledgei* and from open pitchers of *S. flava*. A cube of white of hard-boiled egg, 0.25 inch to the edge, was placed in 5 cc. of pitcher liquor; 0.2 percent of trikresol was used as a bactericide. This substrate was also used in the presence of acid and of alkali.

All experiments in which either carmine fibrin or egg white served as the substrate, were made in vials which were tightly closed with cork stoppers in order to prevent evaporation of their contents.

The action of the pitcher liquor upon the various substrates is described by species in the following paragraphs.

Sarracenia flava

The experiments on *Sarracenia flava*, reported in Table V, demonstrate that a proteolytic enzyme, acting on carmine fibrin, is present in the liquor of closed pitchers as well as in that of open pitchers. They further show that this enzyme is far more active in the presence of 0.2 percent hydrochloric acid than in the presence of 0.5 percent sodium carbonate.

TABLE V.—DIGESTION OF CARMINE FIBRIN BY PITCHER LIQUOR OF *SARRACENIA FLAVA*

Type of pitchers.	*Reaction of medium.*	*Volume of pitcher liquor, cc.*	*Mass of carmine fibrin, gram.*	*Solution of substrate. Marked in hours.*	*Solution of substrate. Complete in hours.*
Closed	0.2% HCl	10	0.20	3	12
		5	0.20	3	15
		5	0.20	3	26
		5	0.20	2.5	17
		5	0.20	<16	<27
		3	0.05	3.5	<14
		3	0.05	8	<14
		4	0.05	7	<14
		3	0.05	5	..
Open	0.2% HCl	10	0.20	2	8
		4	0.05	..	15
		3	0.05	48	96
Closed	0.5% Na_2CO_3	3	0.05	67	76
		4	0.05	..	*
Open	0.5% Na_2CO_3	4	0.05	144	†

The proteolytic enzyme had practically no action on carmine fibrin in the absence of added acid or alkali. In four separate experiments, from 3 to 10 cc. of liquor from *closed* pitchers were permitted to act upon carmine fibrin. In three separate experiments, from 4 to 10 cc. of liquor from *open* pitchers were permitted to act upon that substrate. In each experiment, either 0.05 or 0.20 gram of carmine fibrin was used, with 0.2 percent trikresol as a bactericide. The period of observation varied with the experiment, and ranged from 5 to 42 days. The substrate was not appreciably attacked in any of the seven experiments.

* Solution incomplete at end of 8 days.
† Solution incomplete at end of 40 days.

The edestan test, previously described, was applied to four samples of liquor from closed pitchers and to two samples of liquor from open pitchers. The substrate was completely digested in 1.5 hours by all six samples. A third sample of liquor from open pitchers showed almost complete digestion of the substrate when the period of digestion was extended to 2 hours. In an experiment on liquor from closed pitchers, the substrate was almost all digested on incubation at room temperature for 30 minutes, and was completely digested on incubation at the usual temperature (37.5° C.) for the same period of time.

The casein test, previously described, was also used. On digestion for 2 hours, the casein was partially digested by three of the four samples of liquor from closed pitchers, and by both samples of liquor from open pitchers.

In the experiments with coagulated egg white, four series of tests were made. In two series, only trikresol was added, and incubation occurred at temperatures of 49° to 52° C. and 37.5° C., respectively. In the third series sufficient hydrochloric acid was added to give a concentration of 0.2 percent of that acid, in the fourth series sufficient sodium carbonate to give a concentration of 0.5 percent of that salt; trikresol was used in both series, and incubation occurred in them at a temperature of 37.5° C. In these series, use was made of one composite sample of liquor from each type of pitcher, closed and open. The substrate was not attacked in any of the four series by liquor from either type of pitcher on digestion for a period of 120 hours.

Sarracenia Drummondii and Sarracenia Sledgei

The liquor from both closed pitchers and open pitchers of *Sarracenia Drummondii* and of *Sarracenia Sledgei* contains a protease which digests carmine fibrin in the presence of 0.5 percent sodium carbonate, as may be seen on reference to Table VI. Tests were also made to ascertain the activity of this enzyme in the presence of dilute acid, and also without the addition of either acid or alkali. Each experiment was made on a separate gathering of pitcher liquor.

Six experiments were made on liquor from *closed* pitchers of *S. Drummondii* in the presence of 0.2 percent hydrochloric acid, using 0.05 gram of carmine fibrin and from 2.0 to 4.2 cc. of liquor in each experiment. The period of observation ranged from 12 to 54 days. Four experiments were carried out with liquor from *closed* pitchers of *S. Sledgei* in the presence of 0.2 percent hydrochloric acid; the mass of carmine fibrin ranged from 0.025 to 0.05 gram, the

volume of liquor from 2.0 to 3.8 cc., the period of observation from 12 to 60 days. The substrate was not even partially dissolved in any of these ten experiments.

TABLE VI.—DIGESTION OF CARMINE FIBRIN BY PITCHER LIQUOR OF *SARRACENIA DRUMMONDII* AND *SARRACENIA SLEDGEI* IN AN ALKALINE MEDIUM

Species and Type of Pitcher	*Volume of Pitcher Liquor, cc.*	*Mass of Carmine Fibrin, gram.*	*Concentration of Sodium Carbonate, percent.*	*Solution of Substrate Complete in hours.*
Sarracenia Drummondii Closed	1.9	0.05	0.5	2.5
	3.0	0.01	0.5	3.5
	3.0	0.05	0.5	4.5
	2.0	0.05	0.5	<18
	2.0	0.05	0.5	<18
Sarracenia Drummondii Open	3.5	0.025	0.5	1.5
	3.0	0.01	0.5	<14
	3.0	0.05	0.5	<14
	2.0	0.05	0.5	<18
Sarracenia Sledgei Closed	3.5	0.025	0.5	1.5
	1.6	0.01	0.5	3.5
	2.0	0.05	0.5	<18
	2.0	0.05	0.5	<18
Sarracenia Sledgei Open	3.5	0.05	0.5	<14
	3.5	0.025	0.5	1.5
	2.0	0.05	0.5	5.5
Sarracenia Drummondii Open	3.0	0.01	0.5	<11
	3.0	0.01	0.25	<11
	3.0	0.01	0.125	<11
	3.0	0.01	0.0625	<11
	3.0	0.01	0.03125	36
Sarracenia Drummondii Closed	3.0	0.01	0.5	3.5
	3.0	0.01	0.25	5 days
	3.0	0.01	0.125	32 days *
	3.0	0.01	0.0625	†
	3.0	0.01	0.03125	†

In the experiments with liquor from *open* pitchers of both species in the presence of 0.2 percent hydrochloric acid, the volume of pitcher liquor used ranged from 2.0 to 3.5 cc., the mass of carmine fibrin from 0.025 to 0.05 gram. Four experiments were made with liquor of *Sarracenia Drummondii*, the period of observation ranged from 12 to 57 days; the substrate was not attacked in three of the experiments; in the fourth experiment it was partially dissolved at the end of 31 days and practically completely dissolved at the end of 49 days. Three experiments were made with liquor of *Sarracenia Sledgei*, the period of observation ranging from 5 to 57 days; the carmine fibrin was not attacked in two experiments; in the third experiment it was partially dissolved in 37 days and practically completely dissolved in 57 days.

In those experiments which were made on the pitcher liquor without the addition of either acid or alkali, 0.2 percent trikresol was used as a bactericide,

* Partial solution.
† No solution at end of 50 days.

as in all the other experiments. Six experiments were carried out with liquor from *closed* pitchers of *Sarracenia Drummondii*, using from 1.9 to 3.0 cc. of liquor and 0.05 gram of carmine fibrin. In four experiments, which were under observation for periods of 50 to 55 days, the substrate was not attacked. In one experiment the carmine fibrin was completely dissolved in 7 days; in another experiment it showed marked solution at the end of 21 days. Two experiments were made with liquor from *closed* pitchers of *Sarracenia Sledgei*, using 2.0 cc. of liquor and 0.05 gram of substrate; in one experiment complete solution of the carmine fibrin occurred in 7 days, in the other experiment, marked solution in 21 days.

Four experiments were carried out with liquor from *open* pitchers of *Sarracenia Drummondii*; the volume of liquor ranged from 2.0 to 3.5 cc., the mass of carmine fibrin from 0.01 to 0.05 gram. In one experiment, solution of the substrate had not occurred at the end of 21 days; in the other three experiments, solution slowly occurred, being noticeable at the end of approximately 30 days and practically complete at the end of approximately 50 days.

Three experiments were conducted with liquor from *open* pitchers of *Sarracenia Sledgei*, using from 1.5 to 3.5 cc. of liquor and from 0.01 to 0.05 gram of carmine fibrin. In one experiment, solution of the carmine fibrin had not occurred in 9 days. In the other experiments, the substrate was practically completely dissolved in 37 days and 49 days respectively.

In these experiments, as in all the other experiments, digestion of the substrate did not occur in the controls.

The experiments with carmine fibrin demonstrate that the pitcher liquor of *Sarracenia Drummondii* and *S. Sledgei* contains a protease and that this enzyme acts best in an alkaline medium. The liquor from closed pitchers did not show proteolytic activity in the presence of 0.2 percent hydrochloric acid; and that from open pitchers showed this activity only occasionally and on prolonged digestion. As recorded in a preceding section, the liquor from closed pitchers of these species is distinctly acid to litmus. Probably the native acid plus the hydrochloric acid produced a degree of acidity at which the enzyme was inactive. When neither acid nor alkali was added to the pitcher liquor, the substrate, at times, was dissolved on prolonged digestion.

Since the protease acted best in an alkaline environment, experiments were made in which the concentration of sodium carbonate was varied, ranging from 0.5 to 0.03125 percent, while the mass of carmine fibrin and the volume

of pitcher liquor remained constant. The liquor was obtained from pitchers of *Sarracenia Drummondii*. The results are recorded in the bottom section of Table VI. With liquor from open pitchers, the substrate was completely dissolved during the night, in less than 11 hours, at all concentrations of sodium carbonate from 0.5 to 0.0625 percent, and was completely dissolved in 36 hours when the sodium carbonate had a concentration of 0.03125 percent. With liquor from closed pitchers, the time required for solution of the carmine fibrin increased as the concentration of the sodium carbonate decreased. In fact with the higher dilutions of that salt, even partial solution had not occurred at the end of 50 days. In this connection it should be noted that the acid present in the liquor from closed pitchers exerted a neutralizing effect on the sodium carbonate; the reaction mixtures containing 0.0625 and 0.03125 percent of that salt were respectively neutral and acid to litmus, while the others in the series were alkaline to litmus.

The edestan test and the casein test were applied to two samples of liquor from closed pitchers of *Sarracenia Drummondii*, two samples from closed pitchers of *S. Sledgei*, and one sample from open pitchers of *S. Drummondii*.

In the edestan test, after incubation for 1.5 hours, no digestion of the substrate had been produced by the liquor from closed pitchers, while a slight digestion had been produced by that from open pitchers. The edestan, which had been precipitated by the sodium chloride, was not further digested when incubation was continued for an additional period of 70.5 hours at a temperature of 37.5° C.

In the casein test, that protein was completely digested by all five samples of pitcher liquor on incubation for 2 hours at a temperature of 37.5° C. This test was also applied to a third sample of liquor from closed pitchers of *Sarracenia Drummondii;* the casein was completely digested in 8.5 hours.

Coagulated egg white was also used as a substrate with liquor from *closed* pitchers of both species. Two samples of liquor from *Sarracenia Drummondii* and one sample from *S. Sledgei* were permitted to act on egg white in the presence of trikresol without the addition of either acid or alkali. The action of each sample was studied at two temperatures, 37.5° C. and 49° to 52° C. The substrate was not attacked in any of these tests during a period of 120 hours. Other experiments were made in which the pitcher liquor was permitted to act upon the egg white at a temperature of 37.5° C. in the presence of either dilute acid or dilute alkali. The liquor from *S. Sledgei* had no action

on the substrate on incubation for 120 hours in the presence of 0.2 percent hydrochloric acid. In the presence of 0.5 percent sodium carbonate, it produced marked digestion of the egg white in 24 hours; solution was almost complete in 72 hours and complete in 120 hours. Both samples of liquor from *S. Drummondii* had a proteolytic action on the egg white in the presence of 0.5 percent sodium carbonate; incipient digestion was noted in 24 hours, marked digestion in 48 and 144 hours, and advanced digestion in 168 and 216 hours respectively.

Sarracenia rubra

Opportunity did not occur to procure abundant material for study of the pitcher liquor of *Sarracenia rubra*. Experiments were made with a single sample of liquor from closed pitchers, using 1.3 cc. of liquor and 0.01 gram of carmine fibrin in each experiment. In the presence of 0.5 percent sodium carbonate and trikresol, the substrate was completely dissolved in 2 hours. In the presence of 0.2 percent hydrochloric acid and trikresol, partial solution was noted at the end of 9 days, complete solution at the end of 50 days. These results indicate that the pitcher liquor of this species contains a protease which acts best in an alkaline medium.

Sarracenia minor

Field tests were made on one sample of liquor from closed pitchers and on one sample from open pitchers of *Sarracenia minor*. The action of each sample was studied on carmine fibrin in the presence of (1) 0.2 percent trikresol, (2) trikresol plus 0.2 percent hydrochloric acid, and (3) trikresol plus 0.5 percent sodium carbonate. Neither sample exerted any proteolytic action on the substrate on digestion for 30 days in the presence of trikresol without the addition of either acid or alkali. In the case of the *closed* pitchers, 1.6 cc. of liquor and 0.01 gram of carmine fibrin were used in each test; the substrate was partially dissolved in 6 hours and completely dissolved in 15 hours in the presence of 0.2 percent hydrochloric acid; it was almost completely dissolved in 9 days in the presence of 0.5 percent sodium carbonate. In the case of the *open* pitchers, 3.0 cc. of liquor and 0.05 gram of carmine fibrin were used in each test; the substrate was partially dissolved in 6 hours and completely dissolved in 15 hours in the presence of 0.2 percent hydrochloric acid; it was partially dissolved in 15 hours and almost completely dissolved in 27 hours in the presence of 0.5 percent sodium carbonate.

These results indicate that the pitcher liquor of *Sarracenia minor* (*S. variolaris*) contains a protease which acts best in an acid environment, although it also acts, though less rapidly, in an alkaline medium.

Sarracenia psittacina

Sarracenia psittacina was found in Walton County, Florida, in early May, in some abundance but not of maximum size; the new pitchers for the year were just open or about to open. Although these pitchers were obviously secreting liquor in small amounts, yet it was insufficient in quantity to be collected by means of a fine pipette. An attempt was made to secure a diluted pitcher liquor by introduction of approximately 0.5 cc. of water into each of 50 open pitchers which were free from captures; four hours later, the water was collected by means of a pipette, and the composite sample was used in tests for a protease. Four cc. of the collected water and 0.01 gram of carmine fibrin were used in each test. The reaction mixtures were kept under observation for a period of 4 months. No evidence of digestion was obtained in the presence of 0.2 percent trikresol, or in the presence of that bactericide plus 0.5 percent sodium carbonate, or in the controls. In the presence of trikresol plus 0.2 percent hydrochloric acid, the substrate, which had swollen in the usual manner, gradually decreased in volume, but had not dissolved at the end of 4 months. These results indicate that *Sarracenia psittacina* may possibly secrete into its pitcher cavity a protease which is active in the presence of 0.2 percent hydrochloric acid. However, conclusive experiments with this species must be deferred until it is found growing vigorously in an accessible locality.

Sarracenia purpurea

With respect to the amount of liquor present in its closed pitchers, *Sarracenia purpurea* presents difficulties almost as great as *S. psittacina*. However, a pitcher of *S. purpurea* is occasionally found in which the secretion, usually present as a fine, perspiration-like beading on the inner wall of the pitcher, has collected into drops at its bottom.

Two experiments were made in which open pitchers of living plants were thoroughly flushed by a current of water under pressure, then emptied as completely as possible. From 10 to 15 cc. of water were introduced into each pitcher. After the lapse of a number of days, the water was removed by means of a pipette; and the composite sample was subjected to tests for a

protease, in the presence of acid and of alkali, and without the addition of either reagent.

In one experiment two pitchers were used, and the water remained in them for 15 days. In each test 6 cc. of the collected water was permitted to act upon 0.01 gram of carmine fibrin. The collected water almost completely digested the substrate in 57 days in the presence of 0.5 percent sodium carbonate and trikresol. In the other experiment seven pitchers were used, and the water remained in them for 10 days. Fourteen cc. of the collected water were permitted to act upon 0.20 gram of carmine fibrin in each test. The collected water produced marked digestion of the substrate in 40 hours, and completely dissolved it in 64 hours in the presence of 0.5 percent sodium carbonate and trikresol. The substrate was not dissolved in either experiment within the times stated, in the presence of 0.2 percent hydrochloric acid and trikresol, or in the presence of that bactericide without the addition of either acid or alkali.

Liquor from open pitchers was used in a series of digestion experiments in which carmine fibrin served as the substrate. On reference to Table VII it is seen that the liquor contained a protease which completely dissolved the substrate in a short period of time in the presence of 0.5 percent sodium carbonate and trikresol.

The experiment on May 23 was made on liquor freshly collected from open pitchers which had matured during the preceding season and remained green through the winter. The remaining experiments were made on material from pitchers maturing during the season in which the liquor was collected. On November 15 the liquid contents of the pitchers were frozen to cores of ice which were thawed when the samples were collected; therefore the enzyme had not been destroyed by freezing. These two experiments (May 23 and November 15) indicate the retention of proteolytic activity by the liquor throughout the life of the pitcher.

The digestion experiments made in the presence of 0.2 percent hydrochloric acid and trikresol (Table VII) show that the protease may be slightly active under these conditions.

The experiments of June 30, July 1, and July 14 also were carried out in the presence of 0.2 percent trikresol without the addition of either acid or alkali. The period of observation ranged from 2 to 61 days; the substrate was not even partially dissolved in any of these experiments. All of the ex-

periments on this species thus far described were made on material gathered from strong vigorous plants in Ocean County, New Jersey. Liquor was also collected from smaller and less vigorous open pitchers growing in Tolland County, Connecticut, late in July. Ten cc. of this liquor failed to even partially dissolve 0.01 gram of carmine fibrin in 36 days in the presence of 0.5 percent sodium carbonate and trikresol, or 0.2 percent hydrochloric acid and trikresol, or 0.2 percent trikresol without the addition of either acid or alkali.

TABLE VII.—DIGESTION OF CARMINE FIBRIN BY LIQUOR FROM OPEN PITCHERS OF *SARRACENIA PURPUREA*

Reaction of medium.	*Date of collection.*	*Volume of pitcher liquor, cc.*	*Mass of carmine fibrin, gram.*	*Solution of substrate* Marked in	Complete in
0.5% Na_2CO_3.. .	May 23	25	0.20	..	87 hours
	June 5	2	0.01	72 hours	120 "
	June 30	10	0.05	8 "	48 "
	July 1	3	0.01	24 "	48 "
	July 14	25	0.20	..	42 "
	July 16	8	0.01	..	48 "
	November 15	25	0.20	87 "	135 "
0.2% HCl.....	June 5	2	0.01	42 days	..
	June 30	10	0.05	..	*
	July 1	3	0.01	..	†
	July 14	25	0.20	..	‡
	July 16	8	0.01	47 days	..

Darlingtonia Californica

Study was made of the action of liquor from closed, plugged, and open pitchers of *Darlingtonia californica* on various substrates. Carmine fibrin and fibrin were used most frequently. From 5 to 15 cc. of pitcher liquor and from 0.01 to 0.20 gram of one of these substrates were used in each experiment, with 0.2 percent trikresol as a bactericide. Some tests were made in the presence of 0.2 percent hydrochloric acid, others in the presence of 0.5 percent sodium carbonate, still others without the addition of either acid or alkali. The period of observation was usually 10 days, frequently 30 days, occasionally 40 days. Field experiments were made at atmospheric temperature, laboratory experiments at a temperature of 37.5° C. A total of 57 such experiments were performed—19 with fibrin and 38 with carmine fibrin; no digestion of the substrate was produced by liquor from any of the three types of pitcher.

In the edestan test, the period of digestion was prolonged, ranging from 4 to 7 days. Digestion of the edestan was not produced by liquor from closed pitchers (2 samples), plugged pitchers (2 samples), and open pitchers (1

* No solution in 16 days. † No solution in 61 days.

‡ No solution in 48 hours.

sample). Two other samples of liquor from open pitchers produced a very slight digestion of the edestan.

In the casein test, the period of digestion was prolonged, ranging from 1 to 4 days. No digestion of the substrate was produced by liquor from closed pitchers (1 sample) and plugged pitchers (2 samples). The casein was completely digested by two samples of liquor from open pitchers.

In two field experiments, solid casein was used as a substrate. Several milligrams of casein and 5 cc. of pitcher liquor were permitted to digest in the presence of 0.2 percent hydrochloric acid and trikresol. One experiment was made with liquor from closed pitchers, the other with that from open pitchers. The casein was not dissolved at the end of 18 days.

In another series of field experiments, approximately 0.25 gram of solid casein was suspended in 3 cc. of water and introduced into a closed pitcher which was then plugged in the manner already described. Ten pitchers were treated in this manner. When the pitchers were opened 5 days later, undissolved casein was found at the bottom of each pitcher.

The protean derived from castor bean globulin was also used as a substrate in one experiment on liquor from closed pitchers and in one experiment on that from open pitchers. In each experiment, 2 cc. of a 2 percent solution of the globulin in a 5 percent aqueous solution of sodium chloride were mixed with 2 cc. of pitcher liquor and 0.5 cc. of 0.1 normal hydrochloric acid; and sufficient trikresol was added to make the concentration of that bactericide 0.2 percent. The period of incubation was 6 days at a temperature of 37.5° C. The protean, which was precipitated on addition of the acid, was not even partially dissolved in either experiment.

In certain field experiments, coagulated egg white, raw egg white, raw beef, and cooked beef were introduced into pitchers; and study was made of the action of the pitcher liquor upon them.

Four cubes of white of hard-boiled egg—one-eighth inch to the edge—were introduced into each of ten vigorous pitchers which had just opened. Seven of these pitchers were cut open for examination at the end of 72 hours, the remainder at the end of 144 hours. The cubes were found submerged in the pitcher liquor, unchanged in appearance, and with sharp edges. This procedure was repeated with eight closed pitchers and with eight plugged pitchers. The cubes were removed from the pitcher liquor at the end of 24 hours, and found unaltered.

Raw egg white was diluted with water in the ratio of 1 to 9, and was introduced into ten mature plugged pitchers, 10 cc. of the dilution into each pitcher. Five days later the contents of the pitchers were withdrawn; the liquid was somewhat glairy, and yielded a voluminous precipitate on boiling.

Cubes of raw fresh lean beef—one-eighth inch to the edge—were introduced into eight closed, fourteen plugged, and four open pitchers. The pitchers were cut open from 5 to 7 days later; the cubes had not undergone any disintegration, having retained their shape and size. This entire experiment was repeated with cubes of cooked beef with the same result.

The weight of the evidence, comprised in all the foregoing digestion experiments, is that a protease, secreted by the plant, does not occur in the pitcher liquor of *Darlingtonia californica*. The only evidence of the presence of a protease was the slight digestion of edestan and the complete digestion of casein, both produced by certain samples of liquor from open pitchers in laboratory experiments in which extremely small amounts of substrate were used while the period of incubation was prolonged; it is quite possible that this digestion was produced by enzymes of bacterial origin. For none of the tests for the detection of a protease revealed the presence of such an enzyme in the liquor from either closed or plugged pitchers; and, with the exceptions just noted, none of the tests showed the presence of such an enzyme in the liquor from open pitchers.

Stability of the Protease at Room Temperature

A composite sample of liquor from closed pitchers of *Sarracenia flava* was divided into two portions: one portion was preserved by addition of sufficient trikresol to produce a concentration of 0.2 percent of that bactericide; both portions were then kept at the temperature of the room, and tested at intervals with respect to their action on carmine fibrin in the presence of 0.2 percent hydrochloric acid and trikresol. In each test, use was made of 0.05 gram of substrate. The requisite control tests were also made. The data have been collected in Table VIII. The initial test was made on the day of collection.

The results obtained with the liquor preserved with trikresol demonstrate conclusively that the protease in the pitcher liquor retained its activity for over a year under the conditions stated. It is of interest to note that the liquor, to which no bactericide had been added, also exhibited proteolytic

activity during a like period. These results are in harmony with the occurrence of a protease in the liquor obtained in the spring (May 23) from open pitchers of *Sarracenia purpurea* which had matured during the preceding season and remained green throughout the winter.

TABLE VIII.—DIGESTION OF CARMINE FIBRIN BY PITCHER LIQUOR OF *SARRACENIA FLAVA* KEPT AT ROOM TEMPERATURE

Pitcher Liquor	*Period of keeping, days.*	*Volume of Pitcher Liquor, cc.*	*Solution of Substrate Marked in hours.*	*Solution of Substrate Complete in hours.*
Preserved with trikresol	0	4	7	<18
	18	3	15	24
	34	3	..	21
	54	3	24	<120
	67	3	7	<23
	37°	3	21	46
Without bactericide	0	4	7	<18
	18	3	24	40
	34	3	..	21
	54	3	24	<120
	67	3	7	<23
	37°	3	..	21

Retention of Proteolytic Activity After Dilution of the Pitcher Liquor

It is a matter of common observation that beating rains enter the pitchers of most species of *Sarracenia*, necessarily diluting their normal liquid contents. As is well known, enzymes continue to act on dilution of their solutions. The following experiments were therefore undertaken to ascertain if the protease of the pitcher liquor obeys this general law, and continues to act on dilution of the liquor, a phenomenon which necessarily occurs in nature.

The liquor was collected from pitchers and diluted with sterile water in varying proportions. These dilutions were permitted to act on carmine fibrin in the presence of 0.2 percent trikresol. In each experiment, the reaction (acid or alkaline) was that which had been found most suitable for the protease of that particular species in the experiments already described.

The results, which have been collected in Table IX, demonstrate that the protease of the pitcher liquor retained its activity on dilution of the latter; appreciable masses of carmine fibrin were completely dissolved in a few hours even when the degree of dilution was as great as 1:17.

Stimulation Experiments

Separate studies were made of the influence of mechanical stimulation and of food stimulation upon the proteolytic activity of the pitcher liquor. The technic of stimulation has been described in the preceding paper.

TABLE IX.—DIGESTION OF CARMINE FIBRIN BY DILUTED PITCHER LIQUOR

Species of Sarracenia	*Type of Pitcher.*	*Degree of Dilution of Pitcher Liquor.*	*Volume of Diluted Pitcher Liquor, cc.*	*Reaction.*	*Mass of Substrate, gram.*	*Solution of Substrate. Marked in hours.*	*Solution of Substrate. Complete in hours.*
S. Drummondii	Closed	1:2	3	0.5% Na_2CO_3	0.01	..	3
		1:3	3	"	0.01	..	4
		1:5	3	"	0.01	..	4.5
		1:9	3	"	0.01	..	<18
		1:17	3	"	0.01	..	<18
S. Drummondii	Open	1:3	3	"	0.01	..	<10
		1:5	3	"	0.01	..	<10
		1:9	3	"	0.01	..	<10
S. Drummondii	Open	3:10	10	0.125% Na_2CO_3	0.05	..	<10
		1:10	10	"	0.05	10	<35
S. flava	Closed	1:8	8	0.2% HCl	0.05	6	<18
S. flava	Open	1:8	8	0.2% HCl	0.05	15	48

Mechanical stimulation did not cause the secretion of a protease by *Darlingtonia californica*. With respect to proteolytic activity, liquor of mechanically stimulated pitchers of *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii* did not differ from liquor of unstimulated pitchers of these species.

Attention has already been called to the remarkable response of the pitchers of *Darlingtonia californica* to stimulation by certain *foods* and *reagents*. As a result of the stimulation, the secretion of pitcher liquor was greatly increased. However, a protease was not present in the pitcher liquor of this species after stimulation by the following substances: Sterile milk, raw egg white, coagulated egg white, raw beef, boiled beef, meat broth, fibrin, and acetic acid, hydrochloric acid, and sodium hydroxide in the concentrations stated in the preceding paper.

Summary of Protease Studies

The weight of the evidence is that a protease, secreted by the plant, does not occur in the pitcher liquor of *Darlingtonia californica*.

Sarracenia psittacina possibly secretes a protease active in the presence of dilute acid.

The other species of *Sarracenia* unquestionably secrete proteases. As is shown in one of the following papers, the liquor in closed pitchers is bacteriologically sterile, yet it contains a protease; therefore that enzyme is secreted by the pitcher. Proteolytic activity is exhibited by liquor from both closed pitchers and open pitchers.

The proteases of *Sarracenia flava* and *S. minor* act best in the presence of dilute acid, but also exhibit some activity in the presence of dilute alkali.

The proteases of *S. Sledgei*, *S. Drummondii*, *S. rubra*, and *S. purpurea* act best in the presence of dilute alkali, but also exhibit some activity in the presence of dilute acid. In our experiments, the dilute acid was 0.2 percent hydrochloric acid, the dilute alkali 0.5 percent sodium carbonate. The proteases of *S. Sledgei* and *S. Drummondii* also exhibit some activity without the addition of either acid or alkali.

As is shown in the preceding and following papers, the hydrogen-ion concentration of the liquor from individual pitchers almost always lies on either the acid or the alkaline side of true neutrality. This fact, and the more or less marked activity of the proteases in either an acid or an alkaline environment would indicate that the proteases are active in the liquor in the pitchers, and have a part in the digestion of the prey prior to absorption.

Neither mechanical nor food stimulation of the pitchers increased the proteolytic activity of their liquor.

The protease was active after dilution of the pitcher liquor, thereby duplicating conditions which occur in the native habitat of the Sarracenias.

When the pitcher liquor of *Sarracenia flava* was kept at room temperature, its protease retained its activity for a period of more than a year. The liquor in pitchers of *Sarracenia purpurea* exhibited proteolytic activity during the entire life of the pitchers.

It is of interest to note that proteases have been found in the pitcher liquor of genera of all three families of pitcher plants: *Sarraceniaceæ*, *Cephalotaceæ and Nepenthaceæ*. The work of previous investigators and of ourselves on the *Sarraceniaceæ* is described in this monograph. In previous publications, we have reviewed the work of other investigators on *Nepenthes*, the only genus of the *Nepenthaceæ*, [64, 65] and have described our work on the occurrence of a protease in the pitcher liquor of this genus.[66] A protease, active in the presence of dilute hydrochloric acid, has recently been found by Dakin[67] in the pitcher liquor of *Cephalotus follicularis*, the only genus and species of the *Cephalotaceæ*.

Other Enzymes

Tests for the presence of enzymes, other than proteolytic, were made on composite samples of liquor from pitchers of *Darlingtonia californica* and of *Sarracenia flava*. The pitcher liquor was collected in the field and was mixed with one-tenth its volume of 2.2 percent aqueous solution of trikresol. The sample was shipped to Philadelphia and was examined on its arrival.

The two genera were studied in different years; and all the conditions were not exactly the same in the two series of tests. However, in the tests for urease, diastase, and the inverting enzymes (maltase, emulsin and invertase or sucrase), the ratio of the volume of the pitcher liquor to the concentration of the substrate was kept constant by use of 1.0 cc. of a 1 percent aqueous solution of the substrate for each 1.1 cc. of the sample of pitcher liquor containing trikresol which actually represented 1.0 cc. of the liquor as collected. The temperature of incubation was always 37.5° C. The requisite blank or control experiments were always made using pitcher liquor which had been boiled, then cooled to the temperature of the atmosphere. Whenever necessary, after the pitcher liquor and the substrate had been mixed, sufficient 2.2 percent solution of trikresol was added to bring the concentration of that bactericide in the reaction mixture to 0.2 percent. Urea was used as the substrate for urease, alpha methyl *d*-glucoside for maltase, amygdalin for emulsin, sucrose (cane sugar) for invertase, and soluble starch for diastase. All these reagents were used as 1 percent aqueous solutions prepared by solution of a weighed amount of the substrate in the requisite volume of distilled water at room temperature. Tributyrin was used as the substrate for lipase and ethyl butyrate for esterase. In the tests for diastase and the inverting enzymes, the volume of the composite sample of pitcher liquor used was:—*Darlingtonia californica* 11.00 cc., *Sarracenia flava* 2.75 cc.; these volumes corresponded to 10.00cc. and 2.50 cc. respectively of the liquor as actually collected.

Composite samples of the liquor from closed pitchers of *Darlingtonia californica* and from open pitchers of that species were tested separately for the presence of urease, maltase, emulsin, invertase, and diastase. A composite sample of the liquor from closed pitchers of *Sarracenia flava* was tested for the presence of these five enzymes and of lipase and esterase.

Inverting enzymes.—After incubation for 4 days, both the determination proper and the control were tested for the presence of reducing sugar by means of Benedict's qualitative alkaline copper solution. Neither the determination proper nor the control reduced Benedict's solution in the tests for maltase, emulsin, and invertase in the liquor from closed pitchers of *Darlingtonia californica*, or in the tests for maltase and emulsin in the liquor from open pitchers of that species, or in the tests for maltase and emulsin in the liquor from closed pitchers of *Sarracenia flava*. The determination proper reduced Benedict's solution in the test for invertase in the liquor from open

pitchers of *Darlingtonia californica*, and in the test for that enzyme in the liquor from closed pitchers of *Sarracenia flava*, while the corresponding controls had absolutely no reducing action. The test for invertase was repeated on a second composite sample of liquor from open pitchers of *Darlingtonia californica;* the period of incubation was 12 days; the result was the same as that obtained with the first sample.

These results indicate that the closed pitchers of *Darlingtonia californica* do not contain any of the three enzymes:—maltase, emulsin, or invertase. The liquor in open pitchers of this species does not contain either maltase or emulsin but may contain invertase; the invertase may be of bacterial origin since not present in the liquor of closed pitchers. The liquor in closed pitchers of *Sarracenia flava* does not contain either maltase or emulsin, but does contain invertase.

Diastase.—The period of incubation was as long as 12 days. At intervals, aliquots of both the determination proper and its control were tested for the presence of reducing sugar by means of Benedict's qualitative alkaline copper solution, and for the color reaction with iodine dissolved in an aqueous solution of potassium iodide.

The experiments with the first composite sample of pitcher liquor from closed pitchers of *Darlingtonia californica* were indecisive. After incubation for 10 days, both the determination proper and its control reduced Benedict's solution, and both yielded a blue color with iodine. The test was also applied to a second composite sample of liquor from closed pitchers of this species, the period of incubation being 12 days. The determination proper produced a faint reduction of Benedict's solution, its control no reduction; both gave a blue color with iodine.

With the first composite sample of liquor from open pitchers of *Darlingtonia californica*, after incubation for 4 days, the determination proper gave a slight reduction of Benedict's solution, its control no reduction; after incubation for 10 days, the determination proper gave the reaction of erythrodextrin (red color) with iodine, while its control still gave the blue color produced by starch. A second composite sample of liquor from open pitchers of this species was also tested; the period of incubation was 12 days. The determination proper markedly reduced Benedict's solution, and gave no color with iodine; its control had no action on Benedict's solution and yielded a blue color with iodine.

When the composite sample of liquor from closed pitchers of *Sarracenia flava* was subjected to this test, the result was exactly the same after incubation for 4 days and for 10 days. Neither the determination proper nor its control reduced Benedict's solution, and both yielded a blue color with iodine.

These results indicate that a trace of diastase may be present in the liquor of closed pitchers of *Darlingtonia californica*. Liquor from open pitchers of this species possesses a distinct diastasic activity, but it may be, at least in part, of bacterial origin. The liquor from closed pitchers of *Sarracenia flava* does not contain a diastase.

Urease.--In the tests for the presence of urease, use was made of 11.00 cc. of a composite sample of pitcher liquor from *Darlingtonia californica* or of 5.50 cc. of that from *Sarracenia flava*. These samples had been diluted slightly by addition of trikresol in the field; and the volumes used represented 10.00 cc. and 5.00 cc. respectively of actual pitcher liquor. After the pitcher liquor and the solution of the substrate had been mixed and the proper amount of trikresol solution added, the resulting solution was rendered neutral to methyl orange. After incubation, the solutions were alkaline, and were titrated with 0.1 normal hydrochloric acid using methyl orange as an indicator in order to measure quantitatively the produced alkali.

In the experiments with *Darlingtonia californica*, the period of incubation was 3 days. In each of the four titrations—the determination proper with liquor from closed pitchers and its control and the determination proper with liquor from open pitchers and its control—0.25 cc. of 0.1 normal hydrochloric acid was required to neutralize the alkali.

In the experiment with liquor from closed pitchers of *Sarracenia flava*, both the determination proper and its control required 0.50 cc. of 0.1 normal hydrochloric acid to neutralize the alkali produced during incubation for 4 days.

Since the alkalinity of the determination proper did not exceed that of its control in any of the tests, urease was not present in any of the samples of pitcher liquor examined.

Lipase and *Esterase.*—In testing for the presence of these enzymes in the liquor from closed pitchers of *Sarracenia flava*, 1 cc. of the substrate (tributyrin for lipase, ethyl butyrate for esterase) was mixed with 5.50 cc. of the composite sample of pitcher liquor as collected. The mixture was rendered neutral to phenolphthalein, and was then incubated for 4 days. The liberated butyric

acid was then titrated with 0.1 normal sodium hydroxide using phenolphthalein as an indicator.

In the test for esterase, both the determination proper and its control required 0.10 cc. of 0.1 normal sodium hydroxide in the final titration, therefore esterase was not present.

In the test for lipase, in the final titration with 0.1 normal sodium hydroxide, the determination proper required 0.50 cc. and its control only 0.40 cc. Therefore lipase was probably present.

Summary.—The preceding results lead to the following general conclusions:

The liquor from closed pitchers of *Darlingtonia californica* contained a trace of diastase, while maltase, emulsin, invertase, and urease were absent.

The liquor from open pitchers of *Darlingtonia californica* contained invertase and diastase while maltase, emulsin, and urease were absent. However, no invertase and only a trace of diastase occurred in the liquor from closed pitchers; and these enzymes may have been formed in the open pitchers by bacteria which are present in the contents of open pitchers but are absent from the liquor in closed pitchers.

The liquor from closed pitchers of *Sarracenia flava* contained invertase and probably lipase, while maltase, emulsin, diastase, urease, and esterase were absent.

CHEMICAL CONSTITUENTS OF THE PITCHER LIQUOR OF THE SARRACENIACEÆ

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D., and
FRANK MORTON JONES, F.E.S.

Hydrion Concentration of the Pitcher Liquor

The hydrion concentration of the pitcher liquor was determined in the field by the colorimetric method devised by Wherry [73, 74, 75, 76, 77, 78, 79] for the study of soil reactions. Dr. Wherry had made certain determinations on the liquor of mature pitchers of *Sarracenia purpurea* prior to the inclusion of this phase of the biochemistry of the *Sarraceniaceæ* within the scope of our research. All values, whether determined by him or by ourselves, are expressed as *specific reactions, i.e., specific acidity* or *specific alkalinity*. We are indebted to him for the following results on liquor from open pitchers of various species of *Sarracenia*. Each test, unless otherwise stated, was made on liquor from a single pitcher.

Sarracenia Purpurea

May 8, 1919. Essick Heights, Pa., Bog water acid 300; pitcher liquor acid 300.

May 13, 1919. Green Pond, N. J. Swamp water alkaline 30 to acid 30; spagnum hummocks in which plants grew acid 300; pitcher liquor 5 tests, 1 alkaline 3, 1 neutral, 3 acid 3 to 30.

May 14, 1919. Dover, N. J. Swamp water acid 300; pitcher liquor 3 tests, 3 acid 30 to 300.

June 20, 1919. West Burke, Vt. Bog water acid 300; pitcher liquor acid 300.

August 7, 1919. Nuangola, Pa. Bog water acid 300; pitcher liquor acid 300.

January 1, 1921. Gary, Ind. Bog water alkaline 3; muck at base of plants neutral; pitcher liquor acid 3.

June 28, 1922. Wilmington, N. C. Sandy soil acid 300; pitcher liquor acid 300.

August 20, 1922. Laurel, Md. Swamp soil acid 300; pitcher liquor acid 300.

August 3 to 18, 1923. Mount Desert Island, Me. New Mill Meadow. Stream water acid 3, peat soil acid 30, liquor from young pitcher alkaline 10, liquor from older pitcher of same plant acid 300. Witch Hole. Lake water acid 30, sphagnum soil acid 1,000,

liquor from young, just opened pitcher acid 1,000, old pitcher alkaline 10.

Great Cranberry Island. Peat soil acid 1,000, pitcher liquor 10 tests, acid 100 to 3,000.

September 9, 1923. Mineral Springs, Ind. Swamp water alkaline 3 to 10, soil hummocks neutral to acid 300, pitcher liquor several tests acid 10 to 1,000.

Other Species

S. minor. June 20, 1922. Cox, Ga. Mud soil acid 30, pitcher liquor acid 300. Peat soil acid 300, pitcher liquor acid 300.

June 24, 1922. Summerville, S. C. Woodland soil acid 300, pitcher liquor acid 300.

June 25, 1923. Buffton, S. C. Pitcher liquor acid 1,000.

S. flava. June 25, 1921. Flat Rock, N. C. Sandy meadow soil acid 300; pitcher liquor acid 300.

June 24, 1922. Summerville, S. C. Soil acid 300; pitcher liquor 6 tests, 6 acid 30 to 300.

June 29, 1922. Winter Park, N. C. Peaty meadow soil acid 300; pitcher liquor acid 300.

June 25, 1923. Near Statesville, Ga. Pitcher liquor acid 3,000.

S. rubra. June 29, 1922. Winter Park, N. C. Sandy soil acid 300; the pitchers contained no liquor, distilled water was introduced into them and attained a reaction of acid 300.

June 25, 1923. Near Statesville, Ga. The pitchers contained no liquor; distilled water, used as in the preceding experiment, attained a reaction of acid 300.

S. psittacina. June 25, 1923. Near Statesville, Ga. The pitchers contained no liquor; distilled water was introduced into them and attained a reaction of acid 300.

Our tests on *Sarracenia purpurea*, made at Whitings and Davenport, Ocean County, N. J., showed the following range in reaction. Unless otherwise stated, the pitcher liquor was obtained from large, well developed, open pitchers containing much liquor, and each test was made on liquor from a single pitcher.

November 15, 1919. Bog water acid 300; pitcher liquor 7 tests, 1 alkaline 3, 6 acid 3 to 300.

May 23, 1920. Bog water acid 300; pitcher liquor 10 tests, 10 acid 300 to 1,000.

May 29, 1920. Bog water acid 300; pitcher liquor 5 tests, 5 acid 10 to 1,000.

July 7, 1920. Bog water acid 300; pitcher liquor 6 tests, 2 alkaline 3, 4 acid 10 to 1,000.

September 15, 1920. Bog water acid 300; pitcher liquor 8 tests, 8 acid 300 to 1,000.

The tests in May were made on pitchers of the preceding season, the new pitchers not having yet opened.

Three additional tests were made using newly opened pitchers containing few captures and a small volume of liquor. In all three pitchers, the liquor was alkaline 3 in reaction. A composite sample of liquor from 50 such pitchers was also tested, and found to be neutral (alkaline 1, acid 1). These additional tests seem to indicate that the alkaline reaction is more prevalent in the liquor of newly opened active pitchers, while the reaction is more frequently acid in the liquor of older pitchers.

In *our work* on the southern species, tests were made on the liquor from open pitchers of *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*. The experiments on *S. Sledgei* were made at Biloxi, Miss., May 10, 1921. Using individual pitchers, the reaction of the liquor was neutral in 2 pitchers, alkaline 3 in 1 pitcher, and ranged from acid 3 to acid 10 in 2 pitchers. A composite sample of liquor from 40 pitchers was neutral in reaction. At the time these tests were made, the volume of liquor in each pitcher was very small, and the liquor was diluted with neutral distilled water in the experiments on individual pitchers.

The experiments on *S. flava* were made at De Funiak Springs, Florida, May 19, 1921. The liquor from 5 pitchers, tested separately, was invariably acid; the reaction ranged from acid 100 to acid 3,000.

The experiments on *S. Drummondii* were made at Freeport, Florida, May 22, 1921. Five individual pitchers were tested; the liquor in 3 pitchers had a reaction of acid 3, that in 2 pitchers a reaction of alkaline 3.

Constituents of the Pitcher Liquor

The *nitrogen content* of the liquor has been determined for various types of pitchers of *Darlingtonia californica* and *Sarracenia flava*. Liquor from

open pitchers was filtered prior to analysis. The determination was made by the Gunning modification of the Kjeldahl method. The sample was mixed in a Kjeldahl flask with 10 grams potassium sulphate, a tiny fragment of cupric sulphate, and 25 cc. concentrated sulphuric acid. Heat was applied until the water had been driven off; and the determination was then carried out in the usual manner. The samples were preserved for analysis by addition of trikresol immediately after their collection in the field. The results are reported in Table X.

TABLE X.—TOTAL NITROGEN CONTENT OF PITCHER LIQUOR

Sample Number	*Genus and Species.*	*Type of Pitcher*	*Actual Volume of Pitcher Liquor in Sample cc.*	*Weight of Nitrogen in Sample mg.*	*Weight of Nitrogen in 1 cc. of Pitcher Liquor, mg.*
1.	*Darlingtonia californica*	Closed	45.45	1.26	0.027
2.	*Darlingtonia californica*	Plugged	22.75	0.35	0.015
3.	*Darlingtonia californica*	Plugged	45.45	0.42	0.009
4.	*Darlingtonia californica*	Open	45.45	1.54	0.034
5.	*Darlingtonia californica*	Open	45.45	2.24	0.049
6.	*Sarracenia flava*	Closed	25.00	0.91	0.036
7.	*Sarracenia flava*	Open	50.00	3.01	0.060
8.	*Sarracenia flava*	Open	25.00	1.26	0.050

Gross Composition.—A composite sample of liquor from closed pitchers of *Darlingtonia californica* had a specific gravity of 1.003 at 15° C. It contained 0.213% total solids, 0.104% ash, and 0.046% calcium oxide (lime). The lime formed 44.23% of the ash. The total solids were determined by drying a known mass (approximately 15 grams) of the pitcher liquor, free from preservatives, in a platinum dish at the temperature of boiling water until the weight became constant. The ash was determined by incinerating the total solids in a muffle furnace at a dull red heat. The calcium was precipitated as the oxalate and weighed as the sulphate. Chlorides were present in the liquor, for it yielded a white curdy precipitate with an aqueous solution of silver nitrate; this precipitate dissolved in ammonia water, and was reprecipitated from the resulting solution by nitric acid.

Tests for Reducing Sugar.—Composite samples of liquor from closed pitchers of *Darlingtonia californica* and *Sarracenia Sledgei*, and from open pitchers of *D. californica*, *S. Sledgei* and *S. Drummondii*—5 samples in all were tested with respect to their action on Benedict qualitative alkaline copper solution. This reagent was not reduced by any of the samples; therefore, reducing sugar was not present in the pitcher liquor examined. It is, however, possible that the liquor may at times be contaminated by sugar derived from the nectar.

Presence of Hydrogen Sulphide in the Pitcher Liquor under Certain Conditions

In certain experiments (page 60) on *Darlingtonia californica*, cubes of coagulated egg white were introduced into open pitchers, and examined 144 hours later. The contents of one of the pitchers then had an odor of hydrogen sulphide.

In other experiments on this species, raw egg white was diluted with ninefold its volume of water and introduced into 10 plugged pitchers. Five days later, the contents of these pitchers were collected. A composite sample of the contents was placed in a test tube, acidified with hydrochloric acid, and heated. A piece of filter paper, which had been moistened with an aqueous solution of lead acetate, was held at the mouth of the tube. The evolved gas blackened the paper. Therefore, hydrogen sulphide was present in the contents of these pitchers.

A BACTERIOLOGICAL STUDY OF THE PITCHER LIQUOR OF THE SARRACENIACEAE

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D. and
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Bacteriological studies have been made of the liquor from both closed pitchers and open pitchers of plants growing in their native habitat. Up to date, all the North American *Sarraceniaceae* except *Sarracenia psittacina* have been drawn into the scope of these studies. The liquor from each pitcher was studied separately. The procedure for the collection and laboratory examination of the pitcher liquor was an amplification of that in our study of *Nepenthes*.[68]

Closed Pitchers

Technic. The external surface of the pitcher, near the base of the cavity and slightly above the level of the pitcher liquor, was sterilized by passage through the flame of an alcohol lamp. The pitcher was then cut obliquely through the sterilized region by means of sterile scissors. The upper portion of the pitcher was discarded. The lower portion contained the pitcher liquor, which was immediately poured into a tube containing a "slant" of sterile plain nutrient agar. The usual precautions were observed in making this transfer, *i. e.*, to sterilize the scissors in the flame just before use, to flame the top of the tube and its cotton plug just before removal of the plug to make the transfer, and again before insertion of the plug after making the transfer. In making the transfer by pouring, the pitcher liquor came into contact only with a small area of the inner wall of the pitcher. The tubes were immediately sent to Philadelphia by express.

In a few instances, a sterile platinum loop was used to transfer the pitcher liquor to a tube of sterile gelatin. These tubes were brought to Philadelphia by one of us.

Since closed pitchers of *Sarracenia purpurea* contain so very little liquor, the technic was modified somewhat for this species.

The top of the closed pitcher was passed through the flame of an alcohol lamp. The top was then removed by means of sterile scissors. One cc. of sterile water was immediately introduced into the pitcher cavity by means of a sterile pipette. By imparting a gentle motion to the pitcher, this water

was made to wash the inner wall of the cavity. The water was then transferred, by use of another sterile pipette, to the agar slant.

Upon arrival at Philadelphia, the tubes were incubated for four days at a temperature of 37° C.

A total of 50 experiments were made, each on liquor from a single closed pitcher. These experiments were distributed as follows: *Darlingtonia californica* 3, *Sarracenia minor* 3, *S. Sledgei* 3, *S. flava* 15, *S. Drummondii* 3, *S. rubra* 3, *S. purpurea* 20.

Results. Neither colonies nor other evidence of proliferation of bacteria developed in any of the 50 experiments. Therefore, the pitcher cavity and liquor of closed pitchers is bacteriologically sterile.

Open Pitchers

Technic. The procedure for the transfer of liquor from an open pitcher to a slant of sterile agar was exactly the same as was followed in the case of closed pitchers. However, in the case of open pitchers of *Sarracenia purpurea*, 1 cc. of the liquor was transferred from the pitcher cavity to the agar slant by means of a sterile pipette. A separate pitcher containing prey was used for each experiment.

A total of 39 experiments were made, distributed as follows: *Darlingtonia californica* 3, *Sarracenia minor* 5, *S. Sledgei* 4, *S. flava* 8, *S. Drummondii* 3, *S. rubra* 4, *S. purpurea* 12.

In all 39 experiments, colonies developed upon the agar slants. Therefore, bacteria were always present in the liquor of open pitchers. Sterile physiological (0.85 percent) sodium chloride solution was added to each agar culture, and an emulsion of the bacteria was prepared. This emulsion was then used for the inoculation of the sterile media enumerated below. No effort was made to isolate bacterial species and to test their action in pure culture upon the various media, for the sole purpose of the bacteriological experiments was to permit the bacteria to act on the media in as nearly as possible the same manner as they act on the prey in the pitcher cavity.

The following media were used. The figure in parentheses after each medium shows the number of separate experiments in which it was employed.

I. Substrates for proteolytic bacteria.
   A. Gelatin (38).
   B. Nährstoff-Heyden agar (28).

C. Loeffler blood serum (35).
D. Dorset egg medium (32).
E. Aleuronat-protein agar (6).
F. Casein agar (6).
G. Fibrin agar (3).
H. Ovalbumin agar (3).
I. Litmus milk (28).

II. Substrates for alkali-forming bacteria.
A. Ammonium lactate rosolic acid agar (34).
B. Ammonium tartrate rosolic acid agar (31).
C. Acetamide rosolic acid agar (34).
D. Urea rosolic acid agar (31).
E. Asparagin rosolic acid agar (34).
F. Glycocoll rosolic acid agar (34).

III. Substrates for acid-forming bacteria.
A. Litmus lactose agar (19).
B. Litmus glucose agar (19).

IV. Substrates for the colon-aerogenes group.
A. Lactose bile salt bouillon (49).
B. Trypsinized peptone (28).

The following media are frequently used in routine bacteriological work, and were made in the usual manner: Litmus milk, litmus lactose agar, litmus glucose agar, lactose bile salt bouillon, Dorset egg medium, and Loeffler blood serum. The gelatin[82] and the trypsinized peptone[83] were prepared and used according to the directions of Rivas. The various protein agars and rosolic acid agars were made as suggested by Crabill and Reed.[84] They had as their base a medium containing magnesium and ferrous sulphates, dipotassium phosphate, potassium chloride, and agar. To this base was added either a protein or a simple nitrogenous organic compound, to serve as the sole source of nitrogen and carbon for the bacteria. The various protein agars were made by addition of 1 percent of a protein (Nährstoff-Heyden, aleuronat-protein, casein, fibrin, ovalbumin) to this plain agar prior to sterilization, and thorough suspension of the protein in the sterile mass prior to use. The various rosolic acid agars were made by additions of (*a*) one-half percent by volume of a 2 percent solution of rosolic acid in 60 percent alcohol and (*b*) a simple nitrogenous compound (ammonium lactate or tartrate, acetamide,

urea, asparagin, glycocoll) to the plain agar. One percent of asparagin was used, the other compounds in molecular concentration equal to that of the asparagin. The rosolic acid agars were always sterilized by the discontinuous method. The production of basic compounds (amines or ammonia) by bacteria growing on these media was shown by the red color imparted to the medium beneath and around the colony. Sterile plates of rosolic acid media were always poured, to serve as controls for the determination of the change in color in the experiment proper.

The media, after inoculation with the suspension of the bacteria, were incubated at a temperature of 37° C., and examined at intervals for evidences of bacterial action. Unless otherwise stated, observations were made during a period of 30 days.

Results. The results obtained with each medium may be summarized briefly.

Gelatin. The gelatin was completely liquefied in all 38 experiments. In approximately three-fourths of the experiments, liquefaction was complete by the end of the first week of incubation.

Nährstoff-Heyden agar. Colonies developed in 26 of the 28 experiments. Clearing of the cloudy medium about the colonies occurred in 7 of these experiments, and was apparent on some plates as early as the fifth day of incubation. This clearing of the medium was due to the action of proteolytic bacteria on the Nährstoff-Heyden, which, according to Gotschlich,[85] is a mixture of albumoses (proteoses).

Loeffler blood serum. Digestion of this medium occurred in four-fifths of the 35 experiments. The digestion was slight in 2 experiments and marked in 26 experiments; it became apparent as early as the fifth day of incubation, and tended to become more pronounced as the period of observation lengthened. A putrid odor was evolved in many of the experiments; it became apparent as early as the tenth day of incubation.

Dorset egg medium. Distinct digestion of the medium occurred in 15 experiments. It was noted as early as the fifth day of incubation, and tended to become more marked as the period of incubation increased. Incipient digestion of the medium was observed in 4 additional experiments. Therefore evidence of digestion was present in 59.4 percent of the 32 experiments. A putrid odor developed in many of the experiments, and became apparent as early as the tenth day of incubation.

Aleuronat-protein agar. While colonies developed in all 6 experiments, digestion of the protein with clearing of the medium about the colonies occurred in but 2 experiments.

Casein agar. The bacteria proliferated in all 6 experiments. Digestion of the casein with clearing of the medium about the colonies took place in 3 experiments.

Fibrin agar. Colonies developed in all 3 experiments, but the fibrin was not digested to any appreciable extent.

Ovalbumin agar. Growth of the bacteria occurred in all 3 experiments. However, digestion of the coagulated albumin did not occur to an appreciable extent.

Litmus milk. Coagulation of the milk, *i. e.*, the casein, occurred in all 28 experiments. The coagulum was then digested in 22 experiments; evidence of digestion became apparent as early as the fifth day of incubation; usually from fifty to eighty percent of the coagulum had been dissolved by the end of the period of observation. The litmus was bleached in three-fourths of the experiments.

Rosalic acid agars. The number of experiments in which each of these media was used has been recorded above (see page 77). The entire group of experiments may be summarized in tabular form, reporting for each substrate: (*a*), the percent of experiments in which colonies developed, and (*b*) the percent of experiments in which alkalinity was produced.

	Percent of experiments showing	
Substrate	*Growth*	*Alkalinity*
Ammonium lactate	91.2	88.3
Ammonium tartrate	96.8	83.9
Acetamide	91.2	91.2
Urea	77.4	52.3
Asparagin	97.1	97.1
Glycocoll	94.1	91.2

This alkalinity characterized the colonies and the medium about them, and frequently extended over the entire plate. It could be produced in any one of four ways:—

1. By oxidation of the organic nitrogenous compounds, including the ammonium salts, to ammonium carbonate.

2. By hydrolysis of an amide, *e. g.*, acetamide, and oxidation of the resulting ammonium salt to ammonium carbonate.

3. By desaminization of an amino acid with liberation of ammonia.

4. By decarboxylization of an amino acid with the formation of an amine.

Of the substrates used, acetamide and urea are amides, glycocoll is an amino acid, asparagin is both an amino acid and an amide, and ammonium lactate and tartrate are ammonium salts of organic acids.

A grand total of 198 plates of these rosolic acid agars were inoculated. In 22 percent of them an odor of ammonia or amines developed during the earlier portion of the period of incubation.

Toward the end of this period, a fading or bleaching of the rosalic acid occurred in approximately 40 percent of all the plates inoculated.

Litmus agars. The litmus lactose agar and the litmus glucose agar were used in the same 19 experiments. With the lactose medium, a permanent acidity developed in 2 experiments, a primary acidity followed by a secondary alkalinity in 1 experiment, and a permanent alkalinity in 16 experiments. With the glucose medium, a permanent acidity developed in 4 experiments, a primary acidity followed by a secondary alkalinity in 5 experiments, and a permanent alkalinity in 10 experiments.

These results indicate that the bacterial flora of the liquor in open pitchers attacked the peptone of the medium in preference to its carbohydrate. This was especially true of the lactose medium.

Lactose bile salt bouillon. In 38 experiments, the emulsion of bacteria was sown into lactose bile salt bouillon contained in Dunham fermentation tubes. In each of 11 additional experiments, a 2 cc. sample of liquor from an open pitcher was sown into a sterile tube of this medium in the field, using a sterile pipette to collect the liquor from the pitcher and transfer it to the tube. The inoculated tubes were carried to Philadelphia.

The period of incubation with this medium was 5 days at a temperature of 37° C. The production of gas and its collection in the inner inverted tube (positive reaction) was presumptive evidence of the presence of the colon-aerogenes group of bacteria, commonly called the *Bacillus coli* group.

Of the 49 experiments in which this medium was used, gas was produced in 26 experiments, or 53 percent of the total. The test was always negative (no gas-formation) with liquor from open pitchers of *Darlingtonia californica.* The test was also applied to liquor from open pitchers of each species of *Sarracenia* except *S. psittacina;* with each species, some pitchers yielded positive, some negative results, although they were growing near each other. These

results indicate that the *Bacillus coli* group, if present, is introduced by the captured insects and not from the bog water of the habitat. This conclusion is further supported by the experiments on *Sarracenia purpurea*. The 11 field experiments, mentioned above were made with this species. Two bogs lay on opposite sides of a railroad, and a stream of water flowed through both bogs. Of 7 samples of pitcher liquor, taken in one bog, 5 gave positive and 2 negative results. Of 4 samples, taken in the other bog, all yielded negative results. The liquor from 12 open pitchers growing in these bogs was also studied, using the agar slant technic. The bacterial emulsions thus obtained gave 9 positive and 3 negative tests for the presence of the *B. coli* group. Therefore, of 23 open pitchers in these bogs, 14 (61 percent) presumptively contained members of the colon-aerogenes group while 9 did not. Duplicate samples of water, each containing 2 cc., were taken from the stream, and from each bog in the immediate vicinity of the pitchers whose liquor was used in the field tests. Sterile pipettes were used to collect the water and transfer it to the sterile tubes of the medium. The samples of water and of pitcher liquor were taken at the same time. The stream water and the water of both bogs did not produce gas in the medium. Therefore the organisms of the *B. coli* group, which were present in 61 percent of the *Sarracenia purpurea* pitchers examined, did not come from the surrounding water, and must have been carried into the pitchers by the prey. The absence of this group of micro-organisms from the open pitchers of *Darlingtonia californica* was doubtless due to the very sparsely settled region in which that species grows, and the consequent failure of its prey to become infected by members of the group.

Trypsinized peptone. With this medium, the period of incubation was 5 days at a temperature of 37° C. The test for the presence of indol was then made by means of para-dimethylaminobenzaldehyde and hydrochloric acid. A purplish red color developed if indol had been produced in the medium by the bacteria. A positive reaction for indol was obtained in 21 experiments, *i. e.*, in 75 percent of the 28 experiments in which this medium was used.

Now in these 28 experiments, the lactose bile salt bouillon had also been employed, and gas had been produced in 15 experiments, i. e. in 53.6 percent.

Since indol formation occurred in a higher percent of these experiments than did gas formation, the conclusion may be drawn that indol-forming bacteria, other than members of the *B. coli* group, may be present in the liquor of open pitchers.

General Summary

Closed pitchers. The pitcher cavity and liquor of closed pitchers invariably was bacteriologically sterile.

Open pitchers. The liquor from open pitchers, which had captured prey, invariably contained bacteria. The bacterial flora of this liquor always included species which digest proteins. Gelatin was always liquefied. Loeffler blood serum and the casein of litmus milk were digested in approximately 80 percent of the experiments in which these respective media were used. The proteins of Dorset egg medium were attacked in about 60 percent of the experiments in which it was employed. The proteins in the various protein-agars were not markedly attacked, but supported growth of the bacteria. As a rule, the bacteria from a given open pitcher produced proteolysis of at least 2 or 3 substrates and frequently of 4 or 5 substrates.

However, the bacteria digested the proteins so slowly that their part in the digestion of the prey must be a minor one on the genus *Sarracenia*, the protease of the pitcher liquor playing the leading rôle. The bacteria apparently live in symbiosis with the Sarracenias, drawing their nutriment from the digested insects, and aiding, to a certain extent, in the digestion of the prey. Since the pitcher liquor of *Darlingtonia californica* does not contain a protease, the proteolytic bacteria, present in the open pitchers of this species, must be the chief factor in the digestion of the prey. Doubtless, in the pitchers of both genera (*Sarracenia* and *Darlingtonia*), tissue enzymes of the captured insects participate in the digestion by virtue of their autolytic action.

A putrid odor was frequently produced by the action of the bacteria on the Loeffler blood serum and the Dorset egg medium. This phenomenon recalls the observations concerning the presence of hydrogen sulphide in the pitcher contents of *Darlingtonia californica* several days after egg white had been introduced, see page 73.

The bacteria exhibited a marked tendency to attack protein (peptone) in preference to carbohydrate. This was shown by the production of an alkaline reaction in the litmus agars, especially litmus lactose agar. When the bacteria from a given pitcher produced an alkaline reaction in the litmus agars, they also produced an alkaline reaction in the rosalic acid agars.

The bacteria were able to use amino acids, amides, and the ammonium salts of organic acids as their sole source of nitrogen and carbon, and, as a result, render the reaction alkaline to rosolic acid; and, at times, they produced

an odor of ammonia and amines. As a rule, the bacteria from a given pitcher grew and produced an alkaline reaction in 5 or 6 of the rosolic acid media. An alkaline reaction to rosolic acid means a hydrogen-ion concentration, pH, numerically equal to or greater than 8.0,[86] or a specific alkalinity numerically equal to or greater than 10. However, the pitcher should be able to maintain the normal reaction of its liquor even if basic compounds be produced in its cavity, just as it maintains the normal reaction when dilute acid or dilute alkali is introduced into the cavity (see page 44).

Members of the colon-aërogenes group of bacteria were present in the liquor of over half of the open pitchers. They apparently were introduced into the pitcher cavity by the prey.

Indol-producing bacteria, not members of the colon-aërogenes group, also were frequently present in the liquor of open pitchers.

THE ABSORPTION OF NUTRIENTS IN THE PITCHERS OF THE SARRACENIACEÆ

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The technic used and the results obtained in this part of the research have been described in detail elsewhere.[19] They may be summarized briefly.

Study was made of:

1. The absorption of water from the pitcher cavity of *Darlingtonia californica*, *Sarracenia Sledgei*, *S. flava*, and *S. Drummondii*.

2. The absorption of nitrogenous compounds, such as ammonium chloride, ammonium tartrate, acetamide, urea, asparagin, glycocoll, trypsinized peptone, peptone, and egg albumin, from the pitcher cavity of *Sarracenia Sledgei*, *S. flava*, *S. Drummondii*, and *S. purpurea*.

3. The absorption of certain nitrogenous compounds (acetamide, urea, asparagin, and peptone) from the pitcher cavity of *Sarracenia purpurea* in the presence of a buffer phosphate solution. The buffer prevented escape of volatile nitrogenous compounds from the pitcher cavity in case they were produced prior to absorption.

4. The absorption of neutral phosphates from the pitcher cavity of *Sarracenia purpurea*.

5. The absorption of the lithium-ion from the pitcher cavity of *Sarracenia purpurea*, the tissues of which were shown spectroscopically to be free normally from the element lithium.

In each experiment with water, measurement was made of the volume of water introduced, and of the volume of the pitcher contents at the end of the experiment. In the experiments with nitrogenous compounds, either by themselves or in the presence of a buffer, and in the experiments with neutral phosphates, determination was made of the volume of solution and the mass of solute introduced, and also of the volume of the pitcher contents and the mass of solute present therein at the end of the experiment.

These studies led to the following general conclusions:

1. Water, which was introduced into the pitchers of *Darlingtonia californica* and the Sarracenias, underwent absorption.

2. When an aqueous solution of a nitrogenous compound was introduced into pitchers of the Sarracenias, both the nitrogenous compound and the water were absorbed, but at a different rate; absorption of the nitrogenous compound was usually more rapid than that of the water.

3. When a phosphate buffer was added to the aqueous solution of the nitrogenous compound, the latter was absorbed while the pitcher contents increased in volume.

4. When a neutral phosphate solution was introduced into pitchers of *Sarracenia purpurea*, both the phosphate and the water were absorbed, but at a different rate; absorption of the phosphate was less rapid than that of the water.

5. The percent of the introduced nitrogenous compound or phosphate absorbed usually increased with the period of absorption.

6. When a solution of neutral lithium citrate was introduced into pitchers of *Sarracenia purpurea*, the lithium ion was absorbed.

7. Absorption by the pitchers of substances introduced into their cavities in solution has been demonstrated (a) by the decrease in the nitrogen or phosphate content of the solution, and (b) by appearance in the pitcher tissues of lithium, an element not normally present.

8. These results indicate that the proteolytic products, formed in the pitcher cavity by digestion of the prey, are absorbed by the pitchers and are utilized for the nutrition of the plant. They also indicate that phosphates, and probably other mineral foods, derived from the prey, are absorbed and utilized in like manner.

CHEMICAL COMPOSITION OF THE TISSUES OF THE SARRACENIACEÆ

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Chemical Composition of the Dried Plants

Specimens of each North American species of the *Sarraceniaceæ* were gathered in the native habitat, in the spring or early summer unless otherwise stated. Rhizomes and pitchers of each species were collected at the same time, but were studied separately. Analyses were made of open pitchers of all species, and of closed pitchers of certain species.

The rhizomes were dug, and washed in running water until free from adherent soil. Dead tissues were removed by trimming. The rhizomes were then spread out, and dried in the air.

Closed pitchers were slit to remove the liquor, then spread out and dried in the air. Open pitchers were slit to the base of the cavity; their contents were completely removed by scraping with a blunt instrument, followed by washing in running water. They were then spread out and dried in the air. Each sample contained from 10 to 60 pitchers.

Prior to analysis, each sample was ground in a drug mill until the entire mass passed through a sieve with 20 meshes to the linear inch. The weight of the sample naturally varied with the abundance of the species and, in the case of the pitchers, with their size. The analysis was made according to the methods of the Association of Official Agricultural Chemists.[80] The results of the analyses are presented in Table XI. The water content has been reported as percent of the air-dried tissues, the other constituents as percent of the total solids. Crude fat is a synonym for ether-extract, crude fiber for cellulose. The nitrogen-free extract includes all carbohydrates except cellulose, and all organic acids. The soluble ash is that portion of the total ash which dissolved in boiling water, while the insoluble ash is the portion which was insoluble in that solvent. The alkalinity of the ash was determined by means of tenth normal hydrochloric acid, using methyl orange as an indicator; it has been reported as cc. of normal acid required to neutralize the ash (soluble or insoluble) yielded by 100 grams of total solids.

The following conclusions are based on a study of the results recorded in Table XI.

With two species (*Sarracenia Sledgei* and *S. Drummondii*), closed pitchers and open pitchers were analyzed separately. The open pitchers contained more crude fat, more crude fiber, less crude protein and less total ash than the closed pitchers of the same species.

The pitchers and the rhizomes of each species may be compared with each other. The rhizomes of *Sarracenia purpurea* were gathered at the same time and place as its "early season" pitchers. As a rule, the rhizomes of a given species contained less crude fiber, less crude protein, and more nitrogen-free extractives than the pitchers of the same species.

The insoluble ash was usually less than the soluble ash in the pitchers, while the reverse was true, as a rule, in the rhizomes.

The alkalinity of the insoluble ash was greater than the alkalinity of the soluble ash in the rhizomes of all the species and in the open pitchers of all the Sarracenias.

The nitrogen-free extractives formed more than one-half of the total solids in all but one sample of rhizomes and in all but two samples of pitchers.

The results obtained with the three samples of pitchers of *Sarracenia purpurea* indicate that the chemical composition of the plants depends, in part, on the time of the year at which they are gathered.

Total Solids and Moisture Content of the Fresh Plants

Plants of several southern species of *Sarracenia* were gathered in their native habitat in the spring, and brought to Philadelphia in the live condition. The homeopathic tinctures were then prepared from their pitchers and rhizomes. In the preparation of these tinctures, it was necessary to determine the moisture content of each specimen, by drying a 10 gram sample for 3 hours at a temperature of 100° C. The following results were obtained:—

Pitchers	*Moisture, percent.*	*Total Solids, percent.*
Sarracenia minor	65.10	34.90
Sarracenia flava	80.60	19.40
Sarracenia Drummondii	75.03	24.97
Sarracenia rubra	68.97	31.03
Rhizomes		
Sarracenia minor	58.53	41.47
Sarracenia flava	76.28	23.72
Sarracenia Drummondii	64.58	35.42
Sarracenia rubra	67.50	32.50

TABLE XI.—CHEMICAL COMPOSITION OF THE SARRACENIACEÆ.

	Genus and Species.	Weight of Sample. Grams.	Moisture.	Total Solids.							Alkalinity of Ash.	
				Crude Fat.	Crude Protein.	Crude Fiber.	Ash. Total.	Ash. Soluble.	Ash. Insoluble.	Nitrogen-free Extractives.	Soluble.	Insoluble.
Pitchers, Closed	*Sarracenia Sledgei*	12	5.48	11.61	13.22	17.95	4.15	3.08	1.07	53.07	29.85	28.55
	Sarracenia Drummondii	9.5	6.94	11.58	13.23	17.52	3.77	2.66	1.11	53.90	27.05	22.15
Pitchers, Open	*Darlingtonia californica*	60	5.14	5.04	8.04	21.33	4.36	3.70	0.66	61.23	30.70	16.15
	Sarracenia minor	33	7.56	12.13	7.84	21.96	1.87	1.17	0.70	56.20	13.85	18.60
	Sarracenia Sledgei	24	9.51	13.81	10.50	19.69	2.56	1.71	0.85	53.44	19.00	19.55
	Sarracenia flava	40	7.71	10.38	8.00	30.15	2.13	1.56	0.57	49.34	16.15	20.15
	Sarracenia Drummondii	30	9.09	14.01	10.45	24.78	3.28	2.03	1.25	47.48	19.15	27.85
	Sarracenia rubra	8	5.61	7.80	8.61	23.41	2.03	0.91	1.12	58.15	11.85	13.75
	Sarracenia purpurea early season.	42	7.15	11.72	5.47	20.98	1.91	1.18	0.73	59.92	11.75	19.50
	Sarracenia purpurea end of season.	62	6.44	6.49	7.62	17.26	2.30	1.36	0.94	66.33	6.75	22.35
	Sarracenia purpurea pitchers of preceding year, gathered in spring.	12	9.44	14.35	1.33	17.26	2.37	1.26	1.11	64.69	8.05	30.50
	Sarracenia psittacina	5	5.81	16.87	7.30	18.27	3.12	2.21	0.91	54.44	2.85	18.80
Rhizomes	*Darlingtonia californica*	8	9.83	25.04	7.91	15.57	3.06	1.60	1.46	48.42	14.30	51.00
	Sarracenia minor	35	7.30	21.08	4.72	12.67	2.61	0.28	2.33	58.92	3.45	21.15
	Sarracenia Sledgei	43	7.60	6.63	8.53	15.40	1.75	0.42	1.33	67.69	3.55	21.85
	Sarracenia flava	46	9.46	15.93	8.49	19.65	2.32	0.98	1.34	53.61	8.30	31.90
	Sarracenia Drummondii	57	7.59	10.32	5.86	13.96	3.90	2.30	1.60	65.96	7.15	27.60
	Sarracenia rubra	14	6.20	5.88	5.42	13.54	9.28	0.25	9.03	65.88	3.00	34.00
	Sarracenia purpurea	18	9.02	9.68	3.81	18.10	2.40	0.96	1.44	66.01	4.95	25.95
	Sarracenia psittacina	5	6.28	11.49	6.49	19.71	5.45	0.35	5.10	56.86	4.35	29.75

Moisture is reported as percent of the air-dried tissues, the other constituents as percent of the totals solids, alkalinity of the ash as cc. of normal hydrochloric acid required by the ash in 100 grams of total solids.

In all four species, the pitchers contained more moisture and less total solids than the rhizomes. The tissues of *Sarracenia flava* contained considerably more moisture than those of any of the other three species studied; this was true of both the pitchers and the rhizomes.

Test for Protease in the Rhizome of Sarracenia purpurea

Rhizomes of *Sarracenia purpurea* were gathered at Whitings and Davenport, New Jersey, at the end of May. Separate portions of the cleansed, crushed, freshly gathered rhizomes were extracted for 21 days at room temperature with the following solvents:—

1. Distilled water, containing 0.2 percent trikresol.
2. Hydrochloric acid 0.2 percent solution, containing 0.2 percent trikresol.
3. Sodium carbonate 0.5 percent solution, containing 0.2 percent trikresol.
4. Alcohol 50 percent by volume.

The water, the dilute acid, and the dilute alkali were used in the ratio of 10 cc. of solvent for each gram of crushed rhizome. The 50 percent alcohol was used in the ratio of 4 cc. of solvent for each gram of crushed rhizome this solvent was used since it contained approximately the same percent of alcohol as whisky, the official whisky, *Spiritus Frumenti* of the United States Pharmacopoeia[81] containing not less than 47 percent and not more than 53 percent by volume of ethyl alcohol. Whisky is frequently used as the menstruum for the preparation of household remedies from the Sarracenias.

The extracts were filtered through filter paper. The aqueous extract was a golden yellow; the insoluble residue was black. The 0.2 percent hydrochloric acid yielded a straw yellow extract, and a residue with the color of reddish brown clay. The 0.5 percent sodium carbonate gave a deep brown extract, and a deep purplish black residue. The presence of trikresol, which was used as a bactericide, possibly influenced the colors obtained with these solvents. The alcoholic extract was a reddish brown, the insoluble residue a light brown.

A series of 6 experiments was now made to test for the presence of a protease in the various extracts. In each experiment, 0.2 gram of carmine fibrin was used as the substrate. The period of incubation was 42 days at room temperature. The volume of extract used in each experiment was:—

Experiment 1. Aqueous extract 50 cc.; sufficient sodium carbonate to produce a concentration of 0.5 percent of that salt.

Experiment 2. Aqueous extract 50 cc.; sufficient hydrochloric acid to produce a concentration of 0.2 percent of that acid.

Experiment 3. Extract in 0.5 percent sodium carbonate solution 50 cc.

Experiment 4. Extract in 0.2 percent hydrochloric acid solution 50 cc.

Experiment 5. Alcoholic extract 20 cc.; sufficient sodium carbonate to produce a concentration of 0.5 percent of that salt.

Experiment 6. Alcoholic extract 20 cc.; sufficient hydrochloric acid to produce a concentration of 0.2 percent of that acid.

The trikresol functioned as a bactericide in the first four experiments, while the alcohol acted as a bactericide in the last two experiments.

Absolutely no digestion of the carmine fibrin occurred in any of the experiments. Therefore, a protease was not present in the rhizomes.

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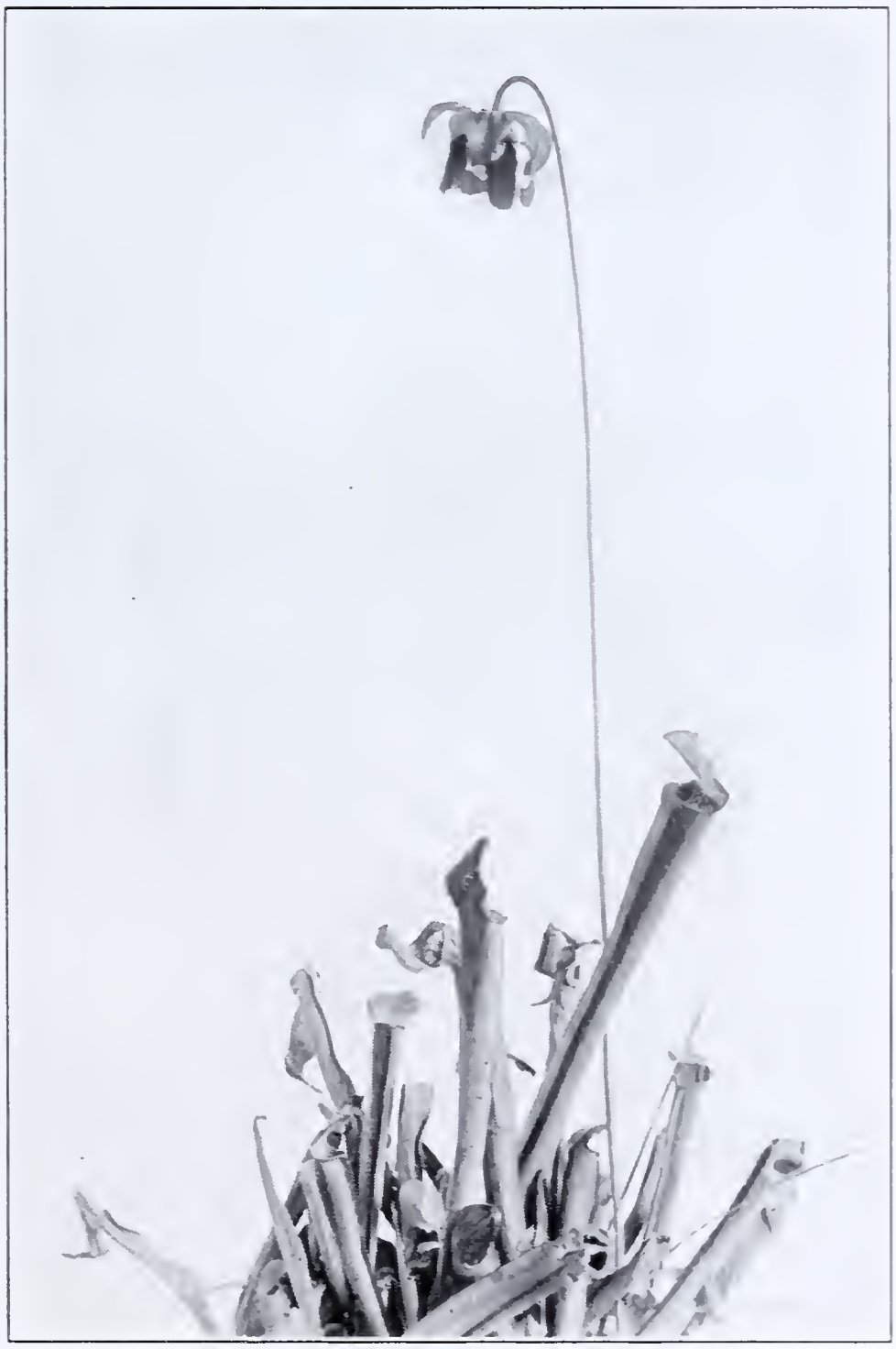
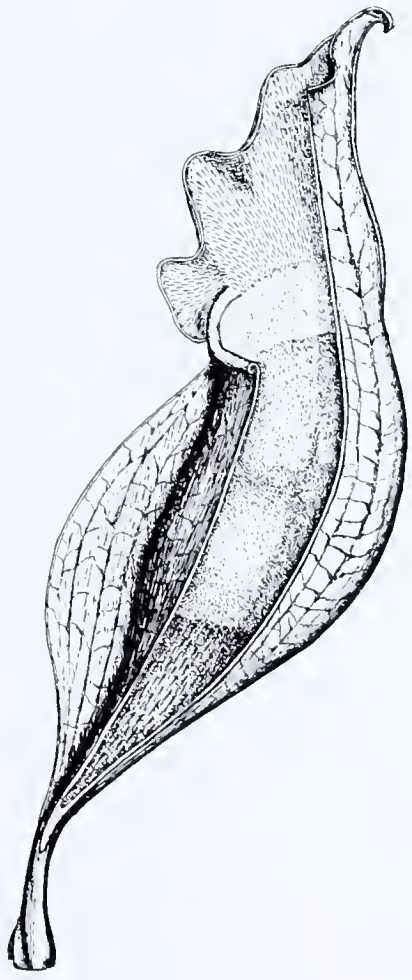


Sarracenia rubra Walt., showing clustered growth and the flower. Pitchers exceeding 60 cm. are not infrequent, but smaller forms, of from 25 to 40 cm., are more common. Photographed at Southern Pines, N. C.

S. drummondii Croom, showing fully opened pitchers and younger forms with closed lids and lips. Mobile Co., Ala., where the mature pitchers attain a height of 45 to 70 cm.

Sarracenia psittacina Michx., showing the blossom and the semi-recumbent pitchers. Photographed in southern Mississippi, where the pitchers commonly attain a length of 20 cm.



Section of open pitcher, *S. purpurea* L., according to Macfarlane, showing wing to the left, the hairy lip, the smooth conducting surface below the rim, the smooth glandular area (a species characteristic) and the detentive zone with its downwardly pointed hairs.

Sarracenia minor Walt. (*S. variolaris* Michx.), showing the hooded pitcher with its translucent windows and its yellow-petalled flower. Photographed at Summerville, South Carolina, where the pitchers of this abundant species commonly reach a height of 30 cm.

Mature plant of *Darlingtonia californica* Torr., showing twisted pitcher and expanded hood, the translucent, window-like spots, and the pendant bilobed flap. Photographed in Plumas Co., Cal., where the pitchers commonly attain a height of 50 to 75 cm.

S. Sledgei Macf., showing recently opened pitchers, the flower, from which the petals have fallen, and the shriveled pitchers of the previous year. Biloxi, Miss., where the pitchers attain a height of 40 to 60 cm.

Group of *S. Flava* L. showing open pitchers. Midsummer at Southern Pines, N. C. The pitchers attain a maximum height of 100 cm., though the usual height is 50 to 75 cm.

S. purpurea L., in full bloom with open pitchers. Ocean County, N. J , where vigorous pitchers often attain a length of 25 or more centimeters

Radiograph of the pitchers of *S. purpurea* L. (full size). The liquid contents have been removed. The mass of captured insects is shown by the shadow. Moths, beetles and flies were identified. Radiograph by Goodspeed.